Methodology for the Development of the 2024-2026 Section 303(d) List in Missouri

Clean Water Commission Approved

October 9, 2024

Missouri Department of Natural Resources
Division of Environmental Quality
Water Protection Program

MISSOURI DEPARTMENT OF NATURAL RESOURCES

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I. Citation and Requirements

A. Citation of the Clean Water Act

The Missouri Department of Natural Resources (MoDNR) is responsible for the implementation and administration of the Federal Clean Water Act (CWA) in Missouri. Pursuant to Section 40 of the Code of Federal Regulations (CFR) 130.7, states, territories and authorized tribes must submit biennially to the United States Environmental Protection Agency (EPA) a list of waters with limited (impaired) water quality, any known pollutants causing the impairments, and the priority ranking of waters targeted for Total Maximum Daily Load (TMDL) development. Under federal regulation of 40 CFR 130.7, states, territories, and authorized tribes are also required to submit to EPA a written methodology document describing the entity's approach in considering, and evaluating existing readily available data used to develop their 303(d) list of impaired water bodies. The Listing Methodology Document (LMD) must be submitted to EPA in the same year as the Section 303(d) list. While EPA does not approve or disapprove the listing methodology, the agency does consider the methodology during its review of the state's 303(d) impaired waters list and its determination to list or not to list waters.

Following approval by the Missouri Clean Water Commission (CWC), the Section 303(d) list, the 305(b) report, and the assessment data on the remaining waters of the state, is submitted to EPA through EPA's Assessment, Total Maximum Daily Load Tracking and Implementation System (ATTAINS). This fulfills Missouri's biennial submission requirement of an integrated report as required under Sections 303(d), 305(b) and 314 of the CWA.

B. U.S. EPA Guidance

In 2001, the Office of General Counsel and the Office of Wetlands, Oceans, and Watersheds developed a recommended framework to assist EPA regions in the preparation of their approval letters for the States' 2002 Section 303(d) list submissions. This was to provide consistency in making approval decisions, along with guidance for integrating the development and submission of the 2002 Section 305(b) water quality reports and Section 303(d) list of impaired waters.[1]

The following sections provide an overview of EPA Integrated Report Guidance documents, available from EPA's website (https://www.epa.gov/tmdl/integrated-reporting-guidance-under-cwa-sections-303d-305b-and-314):

The "2002 Integrated Water Quality Monitoring and Assessment Report Guidance" was the first document EPA provided to the states, territories, and authorized tribes with directions on how to integrate the development and submission of the 2002 Section 305(b) water quality report and Section 303(d) list of impaired waters.

[1] Additional information can be obtained from EPA's website: http://water.epa.gov/lawsregs/lawsguidance/cwa/tmdl/guidance.cfm).

At that time, EPA guidance recommended that states, territories and authorized tribes submit a combined integrated report satisfying the CWA requirements for both the Section 305(b) water quality report and Section 303(d) list. The final 2002 Integrated Report was to include:

- Delineation of water quality assessment units based on the National Hydrography Dataset (NHD);
- Status of and progress toward achieving comprehensive assessments of all waters;
- Water quality standards (WQS) attainment status for every assessment unit;
- Basis for the WQS attainment determinations for every assessment unit;
- Additional monitoring that may be needed to determine WQS attainment status and, if necessary, to support development of TMDLs for each pollutant/assessment unit combination;
- Schedules for additional planned monitoring for assessment units;
- Pollutant/assessment unit combinations still requiring TMDLs; and
- TMDL development schedules that reflect the priority ranking of each pollutant/assessment unit combination.

The 2002 EPA guidance described the requirements of the CWA Section 303(d), under which states, territories, and authorized tribes are required to detail the methodology used in developing their 303(d) list. EPA's guidance recommended states provide: (1) a description of the methodology used to develop Section 303(d) list; (2) a description of the data and information used to identify impaired and threatened waters; (3) a rationale for not using any readily available data and information; and (4) information on how interstate or international disagreements concerning the list are resolved. Lastly (5), EPA recommended that "prior to submission of its Integrated Report, each state should provide the public the opportunity to review and comment on the methodology." In accordance with EPA guidance, the Department continues to review and update the LMD every two years. Once updated, the LMD is made available to the public for review and comment. Following the 60 day public comment period, the Department responds to public comments and provides EPA with a summary of all comments received and Department responses given.

In July 2003, EPA issued new guidance entitled "Guidance for 2004 Assessment, Listing and Reporting Requirements Pursuant to Sections 303(d) and 305(b) of the Clean Water Act." This guidance gave further recommendations about listing of 303(d) and other waters.

In July 2005, EPA published an amended version entitled "Guidance for 2006 Assessment, Listing and Reporting Requirements Pursuant to Sections 303(d), 305(b) and 314 of the Clean Water Act" (US EPA 2005; see Appendix A for excerpt).

In October 2006, EPA issued a memorandum entitled "Information Concerning 2008 Clean Water Act Sections 303(d), 305(b) and 314 Integrated Reporting and Listing Decisions." This memorandum serves as EPA's guidance for the 2008 reporting cycle and beyond. This guidance recommended that each state uses a five-part categorization scheme and provides a

comprehensive description of the WQS attainment status of all segments within the state (reference Table 1). The guidance also defined a "segment" as being synonymous with the term "assessment unit" used in previous Integrated Report Guidance documents. Overall, the selected segmentation approach should be consistent with the state's WQS and be capable of providing a spatial scale that is adequate to characterize the WQS attainment status for the segment.

The 2006 EPA guidance recommended all waters of the state be placed into one of the five categories described below:

Table 1. Placement of Waters within the Five Categories in EPA's Assessment, Listing and Reporting Guidance

Category 1	All designated uses are fully maintained. Data or other information supporting full use attainment for all designated uses must be consistent with the state's LMD. The Department will place a water in Category 1 if the following conditions are met: • The water has physical, chemical (at a minimum, water temperature, pH, dissolved oxygen, ammonia, total cobalt, and total copper for streams; and pH, dissolved oxygen, total nitrogen, total phosphorus, Secchi depth, suspended solids, chlorophyll-a, and algal toxins for lakes), biological, and pathogenic water quality data (at a minimum, *Escherichia coli*, hereafter *E. coli*,) that indicates attainment of WQS. • The level of mercury and other contaminants in fish tissues used for human consumption indicates attainment of WQS. Only samples of higher trophic level species (see Appendix C for list of species) will be used for assessment of mercury. • The water is not rated as "threatened."
Category 2	One or more designated uses are fully attained but at least one designated use has inadequate data or lacks information to make a use attainment decision consistent with the state's LMD. The Department will place a water in Category 2 if *at least one* of the following conditions are met: • There is inadequate data for water temperature, pH, dissolved oxygen, ammonia, total cobalt or total copper in streams to assess attainment with WQS or inadequate data for total nitrogen, total phosphorus, chlorophyll-a, or Secchi depth in lakes. • There is inadequate *Escherichia coli (E. coli)* or fecal coliform bacteria data to assess attainment of the whole body contact recreational use. • There is insufficient fish tissue data available to assess attainment of the fish consumption use. Category 2 waters will be placed in one of two subcategories: **2A.** Waters will be placed in this subcategory if available data, using best professional judgement, <u>suggests compliance</u> with numeric water quality

	criteria in Missouri's WQS (10 CSR 20-7.031) or other quantitative thresholds for determining use attainment. **2B.** Waters will be placed in this subcategory if the available data, using best professional judgment, suggests noncompliance with numeric water quality criteria in Missouri's WQS or other quantitative thresholds for determining use attainment, and these data are insufficient to support a statistical test or to qualify as representative. Category 2B waters will be given high priority for additional water quality monitoring.
Category 3	Water quality data are inadequate to make a use attainment decision consistent with the state's LMD for *any* of the designated uses. The Department will place a water in Category 3 if data are lacking to support a statistical test or to qualify as representative for assessing any of the designated uses. Category 3 waters will be placed in one of two subcategories: **3A.** Waters will be placed in this subcategory if available data, using best professional judgment, suggests compliance with numeric water quality criteria in Missouri's WQS (10 CSR 20-7.031) or other quantitative thresholds for determining use attainment. Category 3A waters will be tagged for additional water quality monitoring but given lower priority than Category 3B waters. **3B.** Waters will be placed in this subcategory if the available data, using best professional judgment, suggests noncompliance with numeric water quality criteria in Missouri's WQS or other quantitative thresholds for determining use attainment. Category 3B waters will be given high priority for additional water quality monitoring.
Category 4	State WQS or other criteria, as per the requirements of Appendix B & C of this document, are not attained but a TMDL exists (4A), a pollution control plan exists (4B), or a pollution control plan is not required (4C). Category 4 waters will be placed in one of three subcategories: **4A.** EPA has approved a TMDL study that addresses the impairment. The Department will place a water in Category 4A if **both** the following conditions are met: • Any portion of the water is rated as being in non-attainment with WQS or other criteria, as explained in Appendix B & C of this document, due to one or more discrete pollutants or discrete properties of the water,[2] and

[2] A discrete pollutant or a discrete property of water is defined here as a specific chemical or other attribute of the water (such as temperature, dissolved oxygen, or pH) that causes designated use impairment and that can be measured quantitatively.

	• EPA has approved a TMDL for the pollutant(s) causing non-attainment. **4B.** Water pollution controls required by a local, state or federal authority are expected to correct the impairment in a reasonable period of time. The Department will place a water in Category 4B if **both** the following conditions are met: • Any portion of the water is rated as being in non-attainment with WQS or other criteria, as explained in Appendix B & C of this document, due to one or more discrete pollutants or discrete properties of water; and • A water quality based permit that addresses the pollutant(s) causing the designated use impairment has been issued, and compliance with the permit limits will eliminate the impairment; or other pollution control requirements have been made that are expected to adequately address the pollutant(s) causing the impairment. This may include implemented voluntary watershed control plans as noted in EPA's guidance document. **4C.** Any portion of the water is rated as being in non-attainment with WQS or other criteria, as explained in Appendix B & C of this document, and a discrete pollutant or discrete property of the water[3] does not cause the impairment.
<u>Category 5</u>	At least one discrete pollutant has caused non-attainment with WQS or other criteria, as explained in Appendix B & C of this document, and the water does not meet the qualifications for listing as either Categories 4A or 4B. *Category 5 waters are those that are candidates for the state's 303(d) list.*[3] If a designated use is not supported and the segment is impaired or threatened, the fact that a specific pollutant is not known does not provide reason for excluding a segment from Category 5. Category 5 waters will be placed in one of two subcategories: **5.** These segments must be listed as Category 5, unless the state can demonstrate that no discrete pollutant(s) causes or contributes to the impairment. Pollutants causing the impairment will be identified through the 303(d) assessment and listing process before a TMDL study is written. The TMDL should be written within the time frame specified by EPA guidance for TMDL development, and as it fits within the state's TMDL prioritization schedule. **5-alt.** A water body assigned to 5-alt is an impaired water lacking a completed TMDL but, because an alternative restoration approach is being pursued,

[3] The proposed state 303(d) list is determined by the Missouri Clean Water Commission. The final list is determined by EPA.

	has been assigned a low priority for TMDL development. This subcategorization increases public transparency that the state is pursuing restoration activities on such waters to achieve attainment of WQS. This subcategory also facilitates tracking alternative restoration approaches of 303(d) listed waters in priority areas.
Threatened Waters	A water body is considered "threatened" when it is currently attaining all designated uses, but the data shows an inverse (time) trend in water quality for a discrete pollutant. In such waters, a statistically significant data trend indicates that the designated use will not be met before the next listing cycle. A threatened water will be treated as an impaired water and be placed in the appropriate category (4A, 4B, or 5).

Although EPA has provided additional guidance since the 2008 cycle, these guidance documents have mainly served to provide minor updates, supplemental information, and continue to encourage states to work on more timely submissions of the 303(d) List and 305(b) Report.

In August 2015, the EPA provided draft guidance that would include a Category 5-alternative (5-alt; reference Table 1). Additional information can be found at EPA's website: https://www.epa.gov/tmdl/alternative-restoration-plans#:~:text=EPA%20has%20created%20an%20optional,provide%20transparency%20to%20the%20public.

Missouri has additional requirements for the LMD in the Code of State Regulations 10 CSR 20-7.050. None of the requirements outlined in 10 CSR 20-7.050 conflict with EPA's guidance.

II. The Methodology Document

A. Procedures and Methods Used to Collect Water Quality Data

- Department Monitoring

  The major purposes of the Department's statewide water quality monitoring program are to:

  - characterize background or reference water quality conditions;
  - better understand daily flow events, seasonal water quality variations, and their underlying processes;
  - characterize aquatic biological communities;
  - assess trends in water quality;
  - characterize local and regional effects of point and nonpoint sources pollutants on water quality;
  - check for compliance with WQS and/or wastewater permit limits; and to
  - support development of mitigative strategies, including TMDLs, to restore impaired waters to attainment of WQS.

- Coordination with Other Monitoring Efforts in Missouri

To maximize efficiency, the Department routinely coordinates monitoring activities with other agencies to avoid overlap, and to give and receive feedback on monitoring design. Data from other sources are used for meeting the same objectives as Department-sponsored monitoring. The data must fit the criteria described in the data quality considerations section of this document. The agencies most often involved are the U.S. Geological Survey (USGS), the U.S. Army Corps of Engineers (USACE), EPA, the Missouri Department of Conservation (MDC), and the Missouri Department of Health and Senior Services (DHSS). The Department also tracks the monitoring efforts of the National Park Service; the U.S. Forest Service; several of the state's larger cities; the states of Oklahoma, Arkansas, Kansas, Iowa, and Illinois; and graduate level research conducted at Missouri universities. For those wastewater discharges where the Department has required instream water quality monitoring, the Department may also use monitoring data acquired by the wastewater dischargers as a condition of discharge permits issued by the Department. In 1995, the Department also began using data collected by volunteers that have passed Volunteer Water Quality Monitoring Program Quality Assurance/Quality Control (QA/QC) tests.

- Existing Monitoring Networks and Programs

The following are water quality monitoring activities presently conducted in Missouri:

1. Fixed Station Network

a) Objective - To better characterize background or reference water quality conditions; to better understand daily flow events, seasonal water quality variations, and their underlying processes; to assess trends; and to check for compliance with WQS.

b) Design Methodology - Sites are chosen based on one of the following criteria:
- Site is believed to have water quality that represents many similarly sized streams in the region due to likeness in watershed geology, hydrology, and land use, as well as an absence of impact from significant point or discrete nonpoint source pollution.
- Site is downstream of a significant point or discrete nonpoint source pollution area.

c) Number of Sites, Sampling Methods, Sampling Frequency, and Parameters:
- MoDNR/USGS cooperative network monitors approximately 70 sites statewide, collecting horizontally and vertically integrated grab samples four to twelve times per year. Samples are analyzed for major ions (e.g., calcium, magnesium, sulfate, and chloride), nutrients (e.g., phosphorus and nitrogen), temperature, pH, dissolved oxygen, specific conductance, bacteria (e.g., *E. coli* and fecal coliform) and flow on all visits; for suspended solids and heavy metals two to four times annually; and for pesticides six times annually at four sites.
- MoDNR/University of Missouri-Columbia's lake monitoring network has monitored about 249 lakes since 1989. More than 75 lakes are monitored each year. Each lake is usually sampled a minimum of four times May through September for nutrients, chlorophyll, Secchi depth (transparency), suspended solids, and algal toxins.
- Routine Department monitoring of finished public drinking water supplies for bacteria and trace contaminants.
- Routine bacterial monitoring for *E. coli* of swimming beaches at Missouri's state parks during the recreational season by the Department's Division of State Parks.
- Monitoring of sediment quality by the Department at up to 20 discretionary sites annually. Sites are monitored for heavy metals (e.g., arsenic, cadmium, copper, lead, mercury, nickel, zinc) or organic contaminants (e.g., polycyclic aromatic hydrocarbons or PAHs).

2. Special Water Quality Studies

a) Objective - To characterize water quality effects from a specific pollutant source area.

b) Design Methodology - These studies are designed to verify and measure contaminants of concern based on previous water quality studies, effluent sampling, or Missouri State Operating Permit applications. These studies employ multiple sampling stations downstream and upstream (if appropriate). If contaminants of concern have significant seasonal or daily variation, the sampling design must account for such variation.

c) Number of Sites, Sampling Methods, Sampling Frequency and Parameters: The Department conducts, or contracts, upwards of 10–15 special studies annually, as funding allows. Each study has multiple sampling sites. The number of sites, sampling frequency and parameters all vary greatly depending on the study. Intensive studies would require multiple samples per site collected over a relatively short time frame.

3. Toxics Monitoring Program

The fixed station network and many of the Department's intensive studies monitor for acute and chronic toxic chemicals.[4] Major municipal and industrial dischargers must monitor for acute and chronic toxicity in their effluents as a condition of their Missouri State Operating Permit.

4. Biological Monitoring Program

a) Objectives - To develop numeric biological criteria (biocriteria) describing fish and aquatic macroinvertebrate "reference" communities in Missouri streams, to implement these criteria within state WQS, and maintain a statewide fish and aquatic macroinvertebrate monitoring program.

b) Design Methodology - Biocriteria development for fish and aquatic macroinvertebrates[5] involves identification of reference streams in each of Missouri's aquatic ecoregions and its 17 Ecological Drainage Units (EDUs), respectively. This process also includes intensive sampling of macroinvertebrate and fish communities to quantify temporal and spatial variations in reference streams within and between ecoregions, as well as to assess the aquatic communities in chemically and physically impaired streams.

c) Number of Sites, Sampling Methods, Sampling Frequency and Parameters: The Department has conducted biological sampling of aquatic macroinvertebrates for many years. Since 1991, the Department's aquatic macroinvertebrate monitoring program has consisted of standardized monitoring of approximately 45 to 55 sites twice annually. In addition, MDC presently has a statewide fish and aquatic macroinvertebrate monitoring program, the Resource Assessment and Monitoring (RAM) Program, designed to monitor and assess the health of Missouri's streams on a rotating basis. This program samples a minimum of 450 random and 30 reference sites every five years.

5. Fish Tissue Monitoring Program

a) Objective - To assess the ecological health of the aquatic system and/or the health of the aquatic biota (usually accomplished via whole fish samples); and to assess the potential risk to human health based on contaminant levels in fish tissue.

[4] As defined in 10 CSR 20-7.031(1)
[5] For additional information visit: http://dnr.mo.gov/env/esp/wqm/biologicalassessments.htm

b) Design Methodology - Sites are chosen based on one of the following criteria:
- Site is believed to have water and sediment quality that represents many similarly sized streams or lakes in the region due to likeness in geology, hydrology, and land use, as well as an absence of impact from significant point or discrete nonpoint source pollution.
- Site is downstream of a significant point or discrete nonpoint source pollution area.
- Site has shown fish tissue contamination in the past.

c) Number of Sites, Sampling Methods, Sampling Frequency and Parameters:
- The Department maintains a fish tissue monitoring program to collect whole fish composite samples[6] at approximately 13 fixed sites. In previous years, this was a cooperative effort between EPA and the Department through EPA's Regional Ambient Fish Tissue (RAFT) Monitoring Program. Each site is sampled once every two years. Preferred species for sampling are Common Carp (*Cyprinus carpio*) or one of the redhorse sucker species (*Moxostoma* sp.). These samples are analyzed for chlorinated hydrocarbon insecticides, polychlorinated biphenyls (PCBs), lead, cadmium, mercury, and fat content.
- The Department, EPA, MDC, and DHSS also sample discretionary sites annually for fish fillet composite samples, fillets from individual fish, or fish tissue plug samples (e.g. muscle biopsy for mercury) Targeted fish species include high trophic level species such as Largemouth Bass (*Micropterus salmoides*), Smallmouth Bass (*Micropterus dolomieu*), Walleye (*Sander vitreus*), or Sauger (*Sander canadensis*), for those contaminants that biomagnify. Benthic feeding species such as catfish species (Ictaluridae), Common Carp (*Cyprinus carpio*), or sucker species (Catostomidae), may also be collected for contaminants that are more associated sediments. Other aquatic species and fish eggs may also be collected at select locations.

6. Volunteer Monitoring Programs

Two major volunteer monitoring programs generate water quality data in Missouri. Data generated from these programs are utilized to inform the statewide 305(b) report on general water quality health, used as a screening level tool to determine where additional monitoring is needed or to supplement other water quality data for watershed planning purposes.

- Lakes of Missouri Volunteer Program (LMVP).[7] This is a cooperative program between the Department, the University of Missouri-Columbia, and volunteers who monitor approximately 137 sites on 66 lakes across the state, including Lake Taneycomo, Table Rock Lake, and several lakes in both the St. Louis and Kansas City areas. Lake volunteers are trained to collect samples for total phosphorus, total nitrogen, chlorophyll,

[6] A composite sample is one in which several individual fish (whole fish in this case) are combined to produce one sample.
[7] For additional program information visit: http://www.lmvp.org/

inorganic suspended sediments, and algal toxins. Data from this program is used by the University as part of a long-term study on the limnology of midwestern reservoirs.

- Volunteer Water Quality Monitoring Program (VWQM).[8] VWQM is an activity of the Missouri Stream Team Program, which is a cooperative project sponsored by the Department, MDC, and the Conservation Federation of Missouri (CFM). Volunteers involved in the VWQM Program monitor water quality of streams throughout Missouri. There are currently over 5,000 Stream Teams and more than 3,600 trained water quality monitors across the state.

  Training for VQWM follows a tiered structure. All volunteers are required to attend an Introductory online education. After completing the Introductory course, volunteers must attend an additional stream side training workshop. Each level of training (1, 2, 3 and CSI) is a prerequisite for the next higher level, as is appropriate. Data generated by volunteers in Levels 2, 3, and the Cooperative Stream Investigation (CSI) Program represent increasingly higher quality assurance. For CSI projects, volunteers have completed a QA/QC workshop, a field evaluation, and have been trained to collect samples following Department protocols. Upon completing Introductory, Level 1, and 2 training, volunteers will have received the basic level training needed to conduct visual stream surveys, stream discharge measurements, biological monitoring, and to collect physical and chemical measurements for pH, conductivity, dissolved oxygen, nitrate, and turbidity.

  The CSI Program uses trained volunteers to collect samples and transport them to laboratories approved by the Department. Volunteers and Department staff work together to develop a monitoring plan. All Level 2, 3, and CSI trained volunteers, are required to attend a validation session every three years to ensure equipment, reagents, and methods meet program standards.

- <u>Identification of All Existing and Readily Available Water Quality Data Sources</u>

  Data Solicitation Request

  Two calendar years prior to the current listing cycle, the Department sends out a request for all available water quality data (chemical, physical, and biological). In the solicitation, the Department requests water quality data from within a time frame of approximately two years prior to and including the current calendar year (up to October 31st of the current year). The data solicitation request is sent to multiple agencies, neighboring states, and organizations. In addition, and as part of the data solicitation process, the Department queries available water quality data from national databases such as the Water Quality Portal[9] consisting of EPA's

[8] For additional program information visit: https://dnr.mo.gov/water/get-involved/volunteer-water-quality-monitoring-program
[9] https://www.waterqualitydata.us/

Water Quality Exchange (WQX) data warehouse,[10] and the USGS National Water Information System (NWIS).[11]

The data must spatially and temporally represent the actual annual ambient conditions of the water body. Sample locations should be characteristic and representative of the main water mass or distinct hydrologic areas. With the exception of data collected for those designated uses that require seasonally based data (e.g., whole body contact recreation, biological community data, and critical season dissolved oxygen), data should be distributed over at least three seasons, over two years, and should not be biased toward specific conditions (such as runoff, season, or hydrologic conditions).

Data meeting the following criteria will be accepted:

- Samples must be collected and analyzed under a QA/QC protocol that follows EPA requirements for quality assurance project plans (QAPPs);
- Samples must be analyzed following protocols that are consistent with EPA or Standard Method procedures;
- All data submitted must be accompanied by a copy of the organization's QA/QC protocol and standard operating procedures;
- All data must be reported in standard units as recommended in the relevant approved methods;
- All data must be accompanied by precise sample location(s), preferably in either decimal degrees or Universal Transverse Mercator (UTM) coordinates;
- All data must be received in a Microsoft Excel or compatible format; and
- All data must have been collected within the requested period of record.
- Fish kills must be verified by agency staff and must have accompanying information such as professional staff observations, water quality samples, meter measurements, or best professional judgment from agency staff as to the cause of the fish kill.

All readily available and acceptable data are uploaded to the Department's Water Quality Assessment (WQA) Database,[12] where the data undergoes quality control checks prior to 303(d) or 305(b) assessment processes.

- Laboratory Analytical Support

The following are laboratories used for each of the various monitoring efforts conducted in Missouri:

- Department/USGS Cooperative Fixed Station Network: USGS Lab in Denver, Colorado

[10] https://www.epa.gov/waterdata/water-quality-data-wqx
[11] https://waterdata.usgs.gov/nwis
[12] https://apps5.mo.gov/mocwis_public/wqa/waterbodySearch.do

- Intensive Surveys: varies, many are completed by the Department's Environmental Services Program (ESP)
- Effluent Toxicity Testing: many commercial laboratories
- Biocriteria for Aquatic Macroinvertebrates: ESP and MDC
- Fish Tissue:Department's Environmental Services Program (ESP), contract laboratories and MDC
- Missouri State Operating Permit: self-monitoring or commercial laboratories
- Department's Public Drinking Water Monitoring: ESP and commercial laboratories[13]
- Other water quality studies: many commercial laboratories

B. Sources of Water Quality Data

The following data sources are used by the Department to aid in the compilation of the state's integrated report (a.k.a the 305(b) report). Where quality assurance programs are deemed acceptable, additional sources would also be used to develop the state's Section 303(d) list. These sources presently include, but are not limited to:

1. Fixed station water quality and sediment data collected and analyzed by ESP personnel.
2. Fixed station water quality data collected by the USGS under contractual agreements with the Department, or organizations other than the Department.
3. Fixed station water quality, sediment quality, and aquatic biological information collected by the USGS under their National Stream Quality Accounting Network and the National Water Quality Assessment Monitoring Programs.
4. Fixed station raw water quality data collected by the Kansas City Water Services Department, the St. Louis City Water Company, the Missouri American Water Company (formerly St. Louis County Water Company), Springfield City Utilities, and Springfield's Department of Public Works.
5. Fixed station water quality data collected by the USACE. The Kansas City, St. Louis, and Little Rock Corps Districts have monitoring programs for Corps-operated reservoirs in Missouri.
6. Fixed station water quality data collected by the Arkansas Department of Environmental Quality, the Kansas Department of Health and Environment, the Iowa Department of Natural Resources, and the Illinois Environmental Protection Agency.
7. Fixed station water quality monitoring by corporations**.**
8. Annual fish tissue monitoring programs by the Department, MDC, DHSS, and EPA.
9. Special water quality surveys conducted by the Department. Most of these surveys are focused on water quality impacts of specific point source wastewater discharges. Some surveys are of well-delimited nonpoint sources, such as abandoned mining areas. Surveys often include physical habitat evaluation and monitoring of aquatic macroinvertebrates, as

[13] For additional information visit: https://dnr.mo.gov/water/business-industry-other-entities/certified-laboratories/chemical; https://dnr.mo.gov/water/business-industry-other-entities/certified-laboratories/microbiological

well as water chemistry.

10. Special water quality surveys conducted by USGS, including but not limited to:
    a) Geology, hydrology and water quality of various hazardous waste and abandoned mining area sites;
    b) Hydrology and water quality of urban nonpoint source runoff in metropolitan areas of Missouri (e.g., St. Louis, Kansas City, Springfield); and
    c) Bacterial and nutrient contamination of streams in southern Missouri.
11. Special water quality studies by other agencies such as MDC, DHSS, and the U.S. Public Health Service.
12. Fish occurrence and distribution monitoring by MDC.
13. Fish Kill and Water Pollution Investigations Reports published by MDC.
14. Selected graduate research projects pertaining to water quality and/or aquatic biology.
15. Water quality, sediment, and aquatic biological data collected by the Department, EPA or their contractors at hazardous waste sites in Missouri.
16. Self-monitoring of receiving streams by cities, sewer districts and industries, or contractors on their behalf, for those discharges that require this kind of monitoring. This effort includes chemical and sometimes toxicity monitoring of some larger wastewater discharges, particularly those that discharge to smaller streams and have the greatest potential to affect instream water quality.
17. Compliance monitoring of receiving waters by the Department and EPA. This can include chemical and toxicity monitoring.
18. Bacterial monitoring of streams and lakes by county health departments, community lake associations, and other organizations using acceptable analytical methods.
19. Other monitoring activities done under a QAPP approved by the Department.
20. Fixed station water quality and aquatic macroinvertebrate monitoring by volunteers who have successfully completed the VWQM Program Level 2 training workshop. Data collected by volunteers who have successfully completed a Level 2 workshop is considered to be Data Code One (see Data Codes below under “Data Type, Amount, and Information Content”). Data generated from VWQM Levels 2, and 3 are considered “screening” level data and can be useful in providing an indication of water quality problems. For this reason, the data are eligible for use in distinguishing between waters in Categories 2A and 2B or Categories 3A and 3B. This data is not used to place waters in main Categories 4 and 5 because analytical procedures do not use EPA approved methods, Standard Methods, or other Department approved methods. Data from volunteers who have not yet completed a Level 2 training workshop do not have sufficient quality assurance to be used in assessment. Data generated by volunteers while participating in the Department’s CSI Program (Section II C1), or other volunteer generated data that otherwise meets the quality assurance outlined in Section II C2 of this LMD, may be used in Section 303(d) assessment.

The following data sources **cannot** be directly used to rate a water as impaired (Categories 4A, 4B, 4C or 5); however, these data sources may be used to target additional monitoring that would allow water quality assessment for Section 303(d) listing:

- Fish Management Basin Plans published by MDC.
- Fish Consumption Advisories published annually by the DHSS. Note: the Department may instead use data from data sources listed above to list individual waters as impaired due to contaminated fish tissue.
- Fish kill reports that have not been verified by state or federal agency professionals.

As previously stated, the Department will review all data of acceptable quality that have been submitted to the Department prior to the public notice of the draft 303(d) list. However, the Department will reserve the right to review and use data of acceptable quality submitted after this date if the new data results in a change to the assessment outcome of a water body.

C. Data Quality Considerations

- DNR Quality Assurance/Quality Control Program

  The Department and EPA Region VII have completed a Quality Management Plan. All environmental data generated directly by the Department, or through contracts funded by the Department, or EPA require a QAPP.[14] The agency or organization responsible for collecting and/or analyzing environmental data must write and adhere to a QAPP approved through the Department's Quality Management Plan. Any environmental data generated via a monitoring plan with a Department approved QAPP are considered suitable for use in water quality assessment and the 303(d) listing. This includes data generated by volunteers participating in the Department's CSI Program. Under this program, the Department's ESP will audit select laboratories. Laboratories that pass this audit will be approved for the CSI Program. Individual volunteers who collect field samples and deliver them to an approved laboratory must first successfully complete Department training on how to properly collect and handle environmental samples. The types of information that allow the Department to make a judgment on the acceptability of a quality assurance program are: (1) a description of the training and work experience of the persons involved in the program, (2) a description of the field meters as well as maintenance and calibration procedures, (3) a description of sample collection as well as handling procedures, and (4) a description of all laboratory analytical methods.

- Other Quality Assurance/Quality Control Programs

  Data generated in the absence of a Department-approved QAPP may be used to assess a water body if the Department determines that the data are adequate after reviewing and accepting the quality assurance procedures plan used by the data generator. This review

[14] For additional information visit: https://www.epa.gov/quality/quality-assurance-project-plan-development-tool

would include: (1) names of all persons involved in the monitoring program, their duties, and a description of their training and work related experience, (2) all written procedures, Standard Operating Procedures, or QAPPs pertaining to the monitoring effort, (3) a description of all field methods used, brand names and model numbers of any equipment, as well as a description of calibration and maintenance procedures, and (4) a description of laboratory analytical methods. This review may also include an audit by the Department's ESP.

- Data Qualifiers

  The Department will handle data qualifiers in different ways depending upon the qualifier, the analytical detection limit, and the numeric WQS:

  - *Less Than Qualifier "<"* - The Department will use half the reported less than value, unless circumstances cause issues with assessment. Examples of this include but are not limited to:
    - Less than values for bacteria. Since the Department calculates a geometric mean, any value less than 1.0 could cause the data to be skewed when using the method of multiplying the values then dividing by the n^{th} root.
    - Less than values below the criterion but still close to the criterion or less than values that are above the criterion. In these cases, the Department will not use the data for assessments.
  - *Non-detection Qualifier "ND"* - The Department treats these the same as the less than qualifier, except for when a value is not reported. For these cases, the Department will use the method detection limit as the reported less than value.
  - *Greater Than Qualifier ">"* - The Department will only consider data with this qualifier for assessments when pertaining to bacteria. In the cases of bacterial data, the reported greater than value is doubled before being used in the assessment calculation. In circumstances where this practice is the sole reason for impairment, the greater than value(s) will be used as the reported value(s) in the assessment calculation (i.e., not doubled).
  - *Estimated Values "E"* - These values, reported as an estimate, are usually characterized as being above the laboratory quantitation limit but below the laboratory reporting limit. High bacteria values are sometimes reported as estimates due to the analytical method used. This usually means the sample had to be diluted during analysis because the true bacteria count was higher than the method reporting maximum. In circumstances where an estimated analytical result is above the laboratory quantification limit but below the laboratory reporting limit, the Department will not use estimated values if the value reported is near the criterion (e.g., a dissolved cadmium result is reported as E0.8 µg/l and the criterion is 0.7 µg/l). If the value is well above or well below the criterion, then it will be used in assessments.

- Data Age

More recent data are preferable for assessing present conditions; however, older data may also be used if the data remains reflective of present conditions.

- If the Department uses data older than **seven years** to make a Section 303(d) list decision, a written justification for the use of such data will be provided.
- If a water body has not been listed previously and all data indicating an impairment is older than seven years, then the water body shall be placed into Category 2B or 3B and prioritized for future sampling.
- A second consideration is the age of the data relative to significant events that may affect water quality. For example, data collected prior to the initiation, closure, or significant change in a wastewater discharge, or prior to a large spill event or the reclamation of a mining or hazardous waste site, may not be representative of present conditions. Even if the data were less than seven years old, such "pre-event" data would not be used to assess present conditions. It could, however, be used to show trends or determine changes in water quality before and after an event.

- Data Type, Amount, and Information Content

EPA recommends establishing a series of data codes that rate data quality by the kind and quantity of data present at a particular location (EPA 1997[15]). The codes are single-digit numbers from one to four, indicating the relative degree of assurance held in a particular environmental dataset. Data Code One indicates the least assurance or the least number of samples or analytes and Data Code Four indicates the greatest. Based on EPA's guidance, the Department uses the following rules to assign code numbers to data:

- Data Code[16] One: All data not meeting the requirements of the other data codes.
- Data Code Two:
  - Chemical data collected quarterly to bimonthly for at least three years; or
  - Intensive studies that monitor several nearby sites repeatedly over short periods of time; or
  - At least three composite or plug fish tissue samples per water body; or
  - At least five bacterial samples collected during the recreational season of one calendar year.
- Data Code Three:
  - Chemical data collected at least monthly for more than three years on a variety of water quality constituents including heavy metals and pesticides; or
  - A minimum of one quantitative biological monitoring study of at least one aquatic assemblage (fish, macroinvertebrates, or algae) at multiple sites,

[15] *Guidelines for the Preparation of the Comprehensive State Water Quality Assessments (305b) and Electronic Updates*, 1997. (http://water.epa.gov/type/watersheds/monitoring/repguid.cfm)
[16] Data Code One is equivalent to data water quality assurance Level One in 10 CSR 20-7.050 General Methodology for Development of Impaired Waters List, subsection (2)(C), Data Code Two is equivalent to Level 2, etc.

multiple seasons (spring and fall), or multiple samples at a single site when data from that site is supported by biological monitoring at an appropriate control site.

- Data Code Four:
  - Chemical data collected at least monthly for more than three years that provides data on a variety of water quality constituents including heavy metals and pesticides, and chemical sampling of sediment and fish tissue; or
  - A minimum of one quantitative biological monitoring study of at least two aquatic assemblages (fish, macroinvertebrates, or algae) at multiple sites.

In Missouri, the primary purpose of Code One data is to provide a rapid and inexpensive method of screening large numbers of water bodies for obvious water quality problems and to determine where more intensive monitoring is needed. In preparation of the state's Integrated Report, data from all four data quality levels are used. Most data are of Data Code One quality, and without Code One data, the Department would not be able to assess a majority of the state's waters.

In general, when selecting water bodies for the Missouri 303(d) list, only Code Two data (or higher) are used, unless the Department can accurately and confidently characterize the problem using Code One data.[17] Code Two data provides a higher level of assurance that a WQS is not being attained and that a TMDL study is necessary. All water bodies placed in Categories 2 or 3 receive high priority for additional monitoring so that data quality is upgraded to at least Data Code Two. Category 2B and 3B waters will be given higher priority than Categories 2A and 3A.

EPA suggests that states use these codes as a way of describing the type of information collected, the frequency of data collection, the spatial/temporal coverage, and the data quality. Missouri has followed this guidance in using the data codes to explain the information type, collection frequency, and spatial/temporal coverage; however, its application differs in regard to data quality. For data quality, the Department reviews the data, as well as the collection methods and laboratory analyses used to generate the data, on a project-specific basis. If the data is of acceptable quality, the Department marks the project and all associated data as QA acceptable. The Department only uses QA acceptable data for assessments, unless the data provides additional corroboration of impairment or attainment status.

- <u>Data Collection Considerations - Dissolved Oxygen and Flow</u>

  In streams, dissolved oxygen is highly dependent on flow and temperature. For stream assessments, dissolved oxygen measurements must be accompanied by flow measurements. Dissolved oxygen must also be measured from the flowing portion of the stream and not be influenced by flooding or backwater conditions.

[17] When a listing amendment or delisting of a 303(d) water is made using only Code One data, a document will be prepared that includes a display of all data and a presentation of all statistical tests or other evaluative techniques that document the scientific defensibility of the data. This requirement applies to all Code One data identified in Appendix B of this document.

D. How Water Quality Data is Evaluated to Determine Impairment Status for 303(d) Listing Purposes

I. Physical, Chemical, Biological and Toxicity Data

During each reporting cycle, the Department and stakeholders review and revise the guidelines for determining water quality impairment. The guidelines, shown in Appendix B & C, provide general rules of data use and assessment. Additionally, Appendix D provides details about specific statistical analyses used in impairment determinations. If trend analysis indicates that presently unimpaired waters will become impaired prior to the next listing cycle, these "threatened waters" will be judged as impaired. Where Missouri's WQS antidegradation provisions apply, those provisions shall be upheld. Numeric criteria have been adopted into state WQS and are used, as described in Appendix B, to make use attainment decisions.

II. Weight of Evidence Approach

When evaluating narrative criteria described in state WQS, the Department will use a weight of evidence analysis for assessing numerical translators not yet adopted into state WQS (see Appendix C). Under the weight of evidence approach, all available information is examined and the greatest weight is given to data providing the "best supporting evidence" for an attainment decision. Determination of "best supporting evidence" will be made using best professional judgment by Department staff that consider factors such as data quality and site-specific environmental conditions. For those analytes with numeric thresholds, the threshold values given in Appendix C will trigger a weight of evidence analysis to determine the existence or likelihood of a use impairment and the appropriateness of proposing a 303(d) listing based on narrative criteria. This weight of evidence analysis will include the use of other types of environmental data when it is available, or collection of additional data to make the most informed use attainment decision. Examples of other relevant environmental data might include physical or chemical data, biological data on fish, aquatic macroinvertebrates (i.e., Fish Index of Biotic Integrity (fIBI) and Macroinvertebrate Stream Condition Index (MSCI), respectively), algal assemblages, fish tissue data, and water or sediment toxicity data.

Biological data will be given greater weight in a weight of evidence analysis for making attainment decisions for aquatic life use and subsequent Section 303(d) listings. Whether or not numeric translators of biocriteria are met is a strong indicator for the attainment of aquatic life use. Moreover, the Department retains a high degree of confidence in an attainment decision based on biological data that is representative of water quality conditions.

When the weight of evidence analysis suggests but does not provide strong scientifically valid evidence of impairment, the Department will place the water body in question in Categories 2B or 3B. The Department will produce a document showing all relevant data

and the rationale for the attainment decision. All such documents will be available to the public at the time of the first public notice for the proposed 303(d) list. Only after full consideration of all comments on the proposed list will a final recommendation be made on the listing of a water body based on narrative criteria.

III. Biological Data

Methods for assessing biological data typically receive considerable attention during the public comment period of development of the LMD. Currently, a defined set of biocriteria[18] are used to evaluate biological data for assessing compliance with WQS. These biocriteria contain numeric thresholds, that when exceeded relative to prescribed assessment methods, serve as a basis for identifying candidate waters for Section 303(d) listing. Biocriteria are based on aquatic macroinvertebrate community data, fish community data, and other appropriate biological indicators.

In general, when interpreting macroinvertebrate data where Stream Habitat Assessment Project Procedure (SHAPP; MoDNR 2016b) scores indicate habitat is less than 75 percent of either reference or appropriate control stream scores and other data indicating impairment by a discrete pollutant is absent, a water body judged to be impaired will be placed in Category 4C. When interpreting fish community data, a provisional multi-metric habitat index called the QCPH1 index is used to identify stream habitat in poor condition. The QCPH1 index separates adequate habitat from poor habitat using a 0.39 threshold value; whereby, QCPH1 scores greater than 0.39 indicate available stream habitat is adequate, and scores less than 0.39 indicate stream habitat is poor. In the absence of other data indicating impairment by a discrete pollutant, impaired fish communities with poor habitat will be placed in Category 4C. Additional information about QCPH1 is provided in the *Considerations for the Influence of Habitat Quality and Sample Representativeness* section.

The sections below describe methods used to evaluate biological data (macroinvertebrate community, fish community, and other biological data). Background information is included on the development and scoring of biocriteria, procedures for assessing biological data, methods used to ensure sample representativeness, and additional information used to aid in assessing biological data, such as the weight of evidence approach.

Aquatic Macroinvertebrate Community Data

The Department conducts aquatic macroinvertebrate assessments to determine macroinvertebrate community health as a function of habitat and water quality. The health of a macroinvertebrate community is directly related to habitat and water quality. Almost all macroinvertebrate evaluations compare the health of the "target" community to the

[18] This refers to Missouri's Water Quality Standards (10 CSR 20-7.031) Section 5 (Specific Criteria) (R) (Biocriteria). Although the Department uses the term "criteria" in association with biological metrics and indices throughout this document, numeric biological criteria have not been promulgated in the rule. This document uses the developed numerical biological metrics and indices as translators for the Biocriteria portion of 10 CSR 20-7.031(5)(R) [3/31/2018].

health of macroinvertebrate communities from reference streams of the same general size and usually the same EDU.

The Department's approach to monitoring and evaluating aquatic macroinvertebrates is largely based on *Biological Criteria for Wadeable/Perennial Streams of Missouri* (MoDNR 2002). This document provides the framework for numeric biocriteria relevant to the protection of aquatic life use for wadeable streams in the state. Biocriteria were developed using wadeable reference streams that occur in specific EDUs, as mapped by the Missouri Resource Assessment Partnership (MORAP; reference Figure 1). For macroinvertebrates, the numeric biocriteria translator is expressed as a multiple metric index referred to as the MSCI. The MSCI includes four metrics: Taxa Richness (TR); Ephemeroptera, Plecoptera, and Trichoptera Taxa (EPTT); Biotic Index (BI); and the Shannon Diversity Index (SDI). These metrics are considered indicators of stream health that change predictably in response to the environmental condition of a stream.

Metric values are determined directly from macroinvertebrate sampling. To calculate the MSCI, each metric is normalized to unitless values of five, three, or one. Each of the unitless values are then summed for a total possible score of 20. MSCI scores fall into three stream conditions:

- Fully Biologically Supporting (16–20),
- Partially Biologically Supporting (10–14), and
- Non-Biologically Supporting (4–8).

Partially and Non-Biologically Supporting streams will be considered impaired and are candidates for Section 303(d) listing.

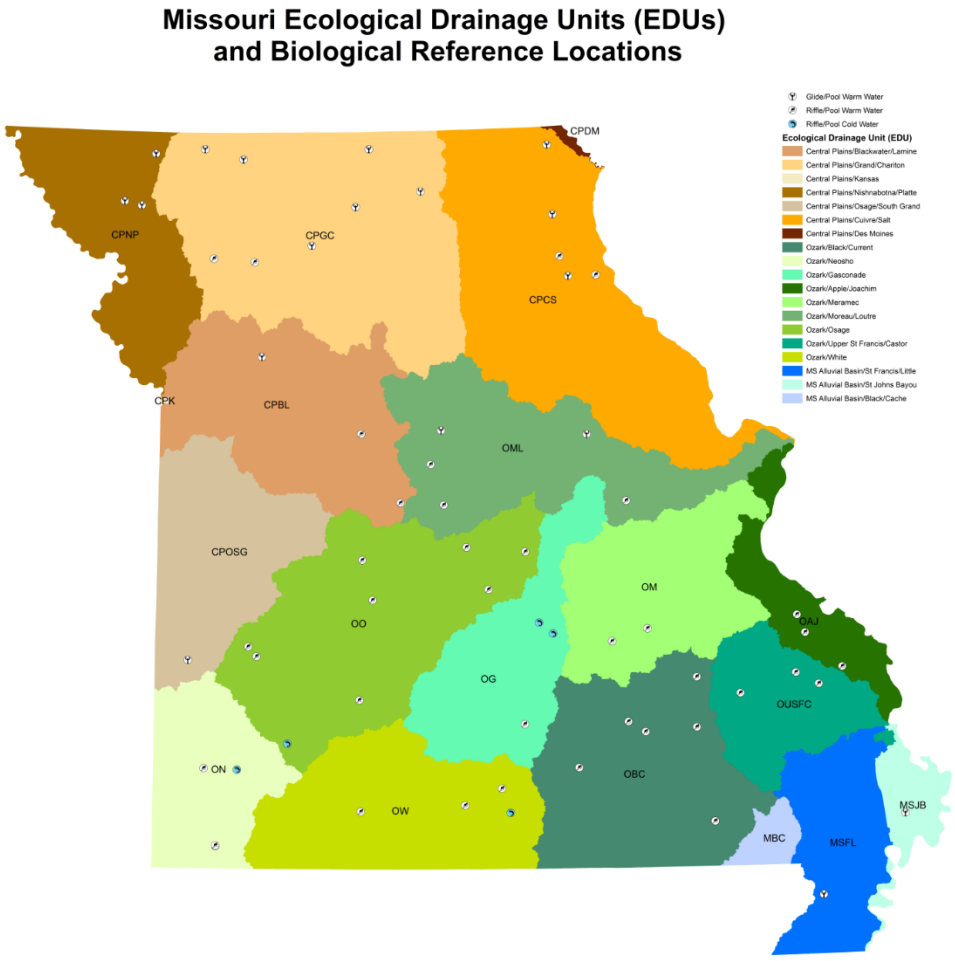

Figure 1: Missouri Ecological Drainage Units (EDUs) and Biological Reference Locations

The unitless metric values (five, three, or one) were developed from the lower quartile of each metric's distribution, as calculated from reference streams for each EDU. The lower quartile (25th percentile) of each metric equates to the minimum value still representing unimpaired conditions. In operational assessments, metric values below the lower quartile of reference conditions are typically judged as impaired (US EPA 1996, Ohio EPA 1990, Barbour *et al.* 1996). Using the 25th percentile of reference conditions as a standard for impairment for each metric provides a conservative estimate of the stream's condition and accounts for natural variability within the stream community.

For metrics with values that decrease with increasing impairment (TR, EPTT, SDI), any value above the lower quartile of the reference distribution receives a score of five. For the BI, whose value increases with increasing impairment, any value below the upper quartile (75th percentile) of the reference distribution receives a score of five. The remainder of each metric's potential quartile range below the lower quartile is bisected and scored either a three or a one. If the metric value is less than or equal to the quartile value and greater than the bisection value, it is scored a three. If the metric value is less than or equal to the bisection value, it is scored a one.

MSCI scores meeting data quality considerations may be assessed for the protection of aquatic life using the following procedures:

Determining Full Attainment of Aquatic Life Use:

- For seven or fewer samples, 75 percent of the MSCI scores must be 16 or greater. Fauna achieving these scores are considered to be very similar to biocriteria reference streams.
- For eight or more samples, results must be statistically similar to representative reference or control streams.

Determining Non-Attainment of Aquatic Life Use:

- For seven or fewer samples, 75 percent of the MSCI scores must be 14 or lower. Fauna achieving these scores are considered to be substantially different from biocriteria reference streams**.**
- For eight or more samples, results must be statistically dissimilar to representative reference or control streams.

Data will be judged inconclusive when outcomes do not meet requirements for decisions of full or non-attainment.

As noted, when eight or more samples are available, results must be statistically similar or dissimilar to reference or control conditions in order to make an attainment decision. To accomplish this, a binomial probability with an appropriate level of significance (α=alpha), is calculated based on the null hypothesis that the test stream would have a similar percentage of MSCI scores that are 16 or greater as reference streams. The significance level is set at α=0.1, meaning if the p-value of the hypothesis test is less than α, the hypothesis is considered statistically significant. The significance level of α is in fact the probability of making a wrong decision and committing a Type I error (rejecting a true null hypothesis). When the Type I error rate is less than α=0.1, the null hypothesis is rejected. Inversely, when the Type I error rate is greater than α=0.1, the null hypothesis is accepted.

For comparing samples from a test stream to samples collected from reference streams in the same EDU, the percentage of samples from reference streams scoring 16 or greater is used to determine the probability of "success" and "failure" in the binomial probability equation. For example, if 84 percent of the reference stream MSCI scores in a particular EDU are 16 or greater, then 0.84 would be used as the probability of success and 0.16 would be used as the probability of failure. Note that Appendix D states to rate a stream as impaired if the frequency of biocriteria reference streams with fully supporting biological scores is greater than five percent more than the test stream, thus, a value of 0.79 (0.84 - 0.05) would actually be used as the probability of success in the binomial distribution equation.

Binomial Probability Example:

Reference streams from the Ozark/Gasconade EDU classified as riffle/pool stream types with warm water temperature regimes produce fully biologically supporting streams 85.7 percent of the time. In the test stream of interest, six out of ten samples resulted in MSCI scores of 16 or more. Calculate the Type I error rate for the probability of getting six or fewer fully biologically supporting scores in ten samples.

The binomial probability formula may be summarized as:

$$p^n + (n!/ X!(n-X)!*p^n q^{n-x}) = 1$$

where,
Sample Size (n) = 10
Number of Successes (X) = 6
Probability of Success (p) = 0.857 - 0.05 = 0.807
Probability of Failure (q) = 0.193

Excel has the BINOM.DIST function that will perform this calculation:

=BINOM.DIST(number_s,trials,probability_s,cumulative)
=BINOM.DIST(6,10,0.807,TRUE)

Using Excel's Binomial Function	
Probability of Success	0.807
Sample Size	10
# of Successes	6
Type 1 Error Rate	0.109

Since 0.109 is greater than the test significance level (minimum allowable Type I error rate) of $\alpha = 0.1$, we accept the null hypothesis that the test stream has the same percent of fully biologically supporting scores as the same type of reference streams from the Ozark/Gasconade EDU. Thus, this test stream would be judged as unimpaired.

If under the same scenario, there were only 5 samples from the test stream with MSCI scores of 16 or greater, the Type I error rate would change to 0.028, and since this value is less than the significance level of $\alpha=0.1$, the stream would be judged as impaired.

Within each EDU, MSCI scores are categorized by sampling regime (glide/pool vs. riffle/pool) and temperature regime (warm water vs. cold water). The percentage of fully biologically supporting scores for the Mississippi River Alluvial Basin/Black/Cache EDU is not available due to the lack of reference sites in this region. Percentages of fully biologically supporting samples per EDU is not included here but can be made available

upon request. The percentage of reference streams per EDU that are fully biologically supporting may change periodically as additional macroinvertebrate samples are collected and processed from reference samples within an EDU.

Sample Representativeness

Field and laboratory methods used by the Department to collect and process macroinvertebrate samples are contained in the document *Semi-Quantitative Macroinvertebrate Stream Bioassessment* (MoDNR 2019). Macroinvertebrates are identified to levels following standard operating procedures contained in *Taxonomic Levels for Macroinvertebrate Identifications* (MoDNR 2022). Macroinvertebrate monitoring is accompanied by physical habitat evaluations, as described in the document *Stream Habitat Assessment* (MoDNR 2019). For the assessment of macroinvertebrate samples, available information must meet Data Code Levels 3 and 4, as described in Section II.C of this LMD. Data coded as Levels 3 and 4 represent environmental data providing the greatest degree of assurance. Thus, at a minimum, macroinvertebrate assessments include multiple samples from a single site, or samples from multiple sites within a single reach.

It is important to avoid situations where poor or inadequate habitat prohibits macroinvertebrate communities from being assessed as fully biologically supporting. Therefore, when assessing macroinvertebrate samples, the quality of available habitat must be similar to that of reference streams within the same EDU. The Department's policy for addressing this concern has been to exclude MSCI scores from an assessment when accompanying habitat scores are less than 75 percent of the mean habitat scores from reference streams of a given EDU. The following procedures outline the Department's method for assessing macroinvertebrate communities from sites with poor or inadequate habitat.

Assessing Macroinvertebrate Communities from Poor or Inadequate Habitat:

- If less than half the macroinvertebrate samples in an assessed stream segment have habitat scores less than 75 percent of the mean score for reference streams in that EDU, any sample that scores less than 16 and has a habitat score less than 75 percent of the mean reference stream score for that EDU, is excluded from the assessment process.
- If at least half the macroinvertebrate samples in an assessed stream segment have habitat scores less than 75 percent of the mean score for reference streams in that EDU and the assessment results in a judgment that the macroinvertebrate community is impaired, the assessed segment will be placed in Category 4C impairment due to poor aquatic habitat.
- If one portion of the assessment reach contains two or more samples with habitat scores less than 75 percent of reference streams from that EDU while the remaining portion does not, the portion of the stream with poor habitat scores could be separately assessed as a Category 4C stream, permitting low MSCI scores.

Macroinvertebrate sampling methods vary by stream type. One method is used in riffle/pool predominant streams, and the other method is for glide/pool predominant streams. For each stream type, macroinvertebrate sampling targets three habitats.

- For riffle/pool streams, the three habitats sampled are flowing water over coarse substrate, non-flowing water over depositional substrate, and rootmat substrate.
- For glide/pool streams, the three habitats sampled are non-flowing water over depositional substrate, large woody debris substrate, and rootmat substrate.

In some instances, one or more of the habitats sampled can be limited or missing from a stream reach, which may affect MSCI scores. Macroinvertebrate samples based on only two habitats may have an MSCI score equal to or greater than 16, but it is also possible that a missing habitat may lead to a decreased MSCI score. Although MoDNR stream habitat assessment procedures take into account a number of physical habitat parameters from the sample reach (e.g., riparian vegetation width, channel alteration, bank stability, bank vegetation protection), they do not exclusively measure the quality or quantity of the three predominant habitats from each stream. When evaluating potentially impaired macroinvertebrate communities, the number of habitats sampled, in addition to the stream habitat assessment score, will be considered to ensure MSCI scores less than 16 are properly attributed to poor water quality or poor or inadequate habitat condition.

Biologists responsible for conducting biological assessments will determine the extent to which habitat availability is responsible for a non-supporting (<16) MSCI score. If it is apparent that a non-supporting MSCI score was due to limited habitat, these effects will be stated in the biological assessment report. This limitation will then be considered when deciding which Listing Methodology Category is most appropriate for an individual stream. This procedure, as part of the Department's biological assessment, will aid in determining whether impaired macroinvertebrate samples have MSCI scores based on poor water quality conditions or habitat limitations.

To ensure assessments are based on representative macroinvertebrate samples, samples collected during or shortly after prolonged drought, shortly after major flood events, or any other conditions that fall outside the range of environmental conditions under which reference streams in the EDU were sampled, will not be used to make an attainment decision for a Section 303(d) listing or any other water quality assessment purposes. Sample "representativeness" is judged by Water Protection Program (WPP) staff after reading the biomonitoring report for that stream, and if needed, consulting with biologists from the Department's ESP. Regarding smaller deviations from "normal" conditions, roughly 20 percent of reference samples failing to meet a fully biologically supporting MSCI score were collected following weather or climatic extremes; as a result, biocriteria for a given EDU are inclusive of samples collected during not only ideal macroinvertebrate rearing conditions, but also during the weather extremes that Missouri experiences.

Assessing Small Streams

Occasionally, macroinvertebrate monitoring is needed to assess streams smaller than the typical wadeable/perennial reference streams listed in Table I of Missouri's WQS. Smaller streams may include Class C streams (streams that may cease flow in dry periods but maintain permanent pools which support aquatic life) or those that are unclassified. Assessing small streams involves comparing test stream and candidate reference stream MSCI scores first to Wadeable/Perennial Reference Stream (WPRS) criteria, and then second to each other.

In MoDNR's Biological Criteria Database, there are 16 candidate reference streams labeled as Class P, 23 labeled as Class C, and 24 labeled as Class U. In previous work by MoDNR, when the MSCI was calculated according to WPRS criteria, the failure rate for candidate reference streams was 31 percent for Class P, 39 percent for Class C, and 70 percent for Class U. The data trend showed a higher failure rate for increasingly smaller high-quality streams when scored using WPRS biological criteria. This trend demonstrates the need to include the utilization of candidate reference streams in biological stream assessments.

Prior to the 2014 revision of the Missouri WQS there was no size classification for streams. The 2014 revision codified size classification for rivers and streams based on five size categories for Warm Water, Cool Water, and Cold Water Habitats. The size classifications are defined as Headwater, Creek, Small River, Large River and Great River. Water permanence continues to be classified as Class P (streams that maintain permanent flow even in drought periods); Class C (streams that cease flow in dry periods but maintain permanent pools which support aquatic life); and the newly adopted Class E (streams that do not maintain permanent surface flow or permanent pools, but have surface flow or pools in response to precipitation events).

Table I of Missouri's WQS lists 62 wadeable/perennial reference streams that provide the current basis for numeric biocriteria. Wadeable/perennial reference streams are a composite of Creek and Small River size classes. Interpretation of Creek (Size Code 2) and Small River (Size Code 3) is based on the MORAP Shreve Link number found in Table 2. These wadeable/perennial reference streams were selected prior to the 2014 revision of the Missouri WQS and were based on the former Table H (Stream Classifications and Use Designations). All, or a portion, of seven wadeable/perennial reference streams are Class C; and all, or a portion, of 57 wadeable/perennial reference streams are Class P.

As part of the 2014 revision of the Missouri WQS, classified streams were changed from only waters listed in Table H to include a modified version of the 1:100,000 National Hydrography Dataset (NHD). This dataset provides a geospatial framework for classified streams and is referred to as the Missouri Use Designation Dataset (MUDD). The streams and rivers now listed in MUDD contain approximately 100,000 miles of newly classified streams, many of which are the Headwater size class. Interpretation of Headwater size (Size Code 1) is based on the MORAP Shreve Link number found in Table 2.

Table 2. Missouri Resource Assessment Partnership Shreve Link Number for Stream Size Code

Stream Size	Size Code	Plains Shreve Link Number	Ozark Shreve Link Number
Headwater	1	1-2	1-4
Creek	2	3-30	5-50
Small River	3	31-700	51-450
Large River	4	701-maximum	451- maximum
Great River	5	Missouri & Mississippi	Missouri & Mississippi
Unknown	0		

In natural channels, biological assessments will be based on criteria established from comparable stream size and permanence. Current WPRS criteria and the MDC fIBI metrics only apply to Creek and Small River size categories. MDC fIBI metrics apply exclusively to the Ozarks ecoregion. Biocriteria have not been established for the size categories of Great River, Large River, or Headwater. The need for alternate criteria for Headwater size class streams is supported by the higher failure rate (70 percent) for small size streams when scored using wadeable/perennial reference stream biocriteria (MoDNR, unpublished data). Since headwater stream biocriteria have not been established, the utilization of candidate headwater reference streams and draft criteria will be necessary to perform biological stream assessments of headwater size streams until scientifically defensible criteria have been developed (Figure 2).

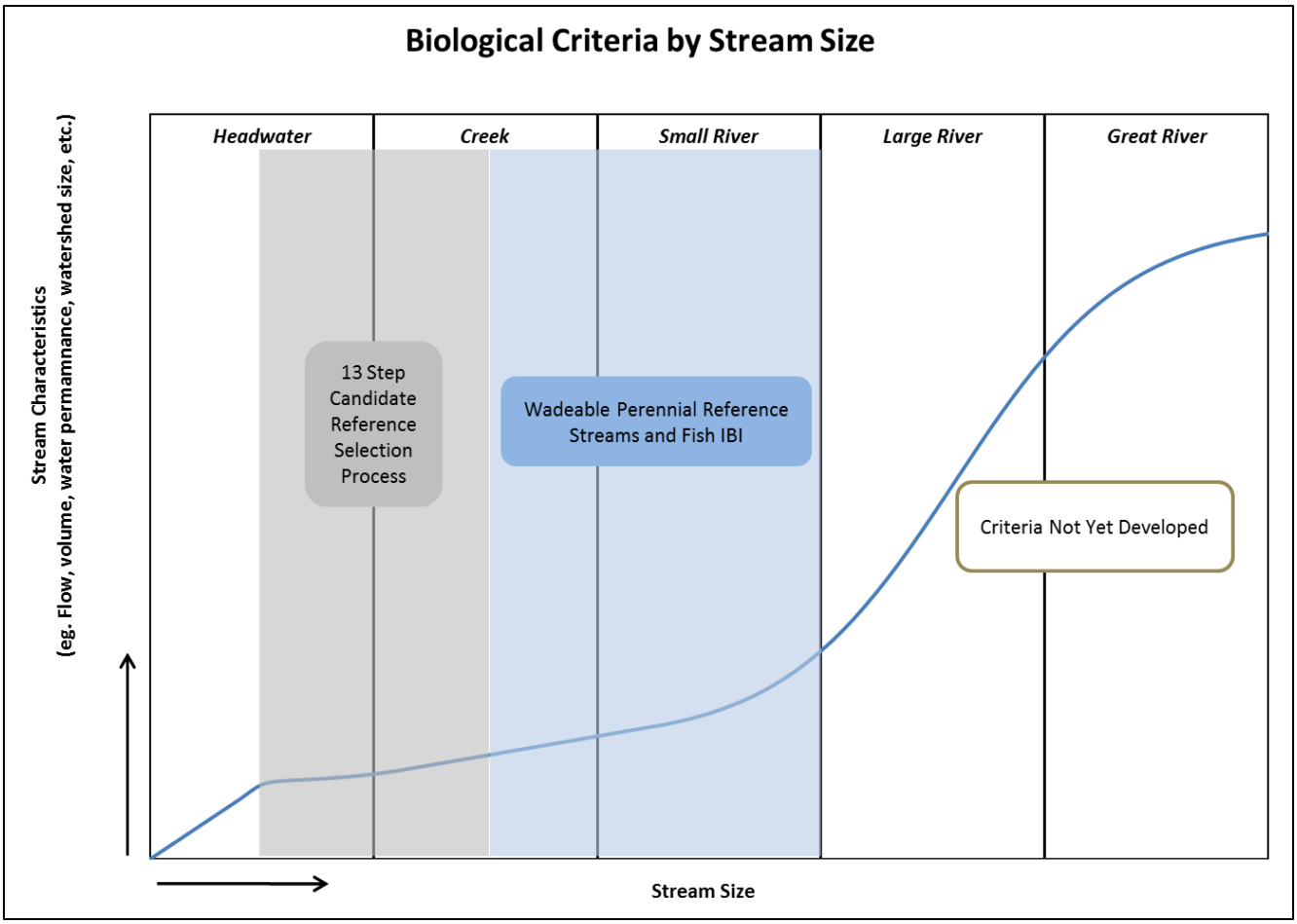

Figure 2. Biological criteria based on stream size classification.

For test streams that are smaller than wadeable perennial reference streams, the Department samples five candidate reference streams of same or similar size and Valley Segment Type (VST) in the same EDU twice during the same year the test stream is sampled (additional information about selecting small control streams is provided below). Although in most cases the Department samples small candidate reference streams concurrently with test streams, existing data may be used if a robust candidate reference stream dataset exists for the EDU.

If the ten small candidate reference stream scores (spring and fall scores from 5 candidate reference streams) are similar to wadeable perennial reference stream criteria, then they and the test stream are considered to have a Class C or Class P warm water habitat designated use, and the MSCI scoring system in the LMD should be used. If the small candidate reference streams have scores lower than the wadeable perennial reference streams, the assumption is that the small candidate reference streams, and the test stream, represent designated uses related to stream size that are not yet approved by EPA in the state's WQS.

The current assessment method for test streams that are smaller than reference streams is stated below.

- If 75 percent of the ten candidate reference stream scores are 16 or greater when compared to WPRS criteria, then the test stream will be assessed using MSCI based procedures in the LMD.

- If 75 percent of the ten candidate reference stream scores are below 16 when compared to WPRS criteria then:

    a) The test stream will be judged "unimpaired" if test stream scores meet criteria developed from the candidate reference stream scores. If 75 percent of the test stream scores are 16 or greater when compared to criteria developed from the candidate reference streams, the stream will be judged "unimpaired."
    b) The test stream will be assessed as having an "impaired" macroinvertebrate community if test stream scores do not meet criteria developed from the candidate reference stream scores. If 75 percent of the test stream scores are less than 16 when compared to criteria developed from the candidate reference streams, the stream will be judged "impaired."
    c) The test stream will be judged "inconclusive" if the requirements in a) and b) are not met.

All work will be documented on the macroinvertebrate assessment worksheet and be made available during the public notice period.

Selecting Small Candidate Reference Streams

Accurately assessing streams that are smaller than reference streams begins with properly selecting small candidate reference streams. Candidate reference streams are smaller than WPRS streams and have been identified as "best available" reference stream segments in the same EDU as the test stream according to watershed, riparian, and in-channel conditions. The selection of candidate reference streams is consistent with framework provided by Hughes *et al.* (1986) with added requirements that candidate reference streams must be from the same EDU and have the same or similar values for VST parameters. If candidate reference streams perform well when compared to WPRS, then test streams of similar size and VST are expected to do so as well. VST parameters important for selection are based on temperature, stream size, flow, geology, and relative gradient, with emphasis placed on the first three parameters.

The stepwise process for candidate reference stream selection is listed below. The Department will fully document its decision-making rationale regarding the steps in this process which will be available upon request and will include but are not limited to: GIS layers used, segment IDs eliminated at the various steps, candidate stream list for field verification, etc.

1. Determine test stream reaches to be assessed. *Missouri Department of Natural Resources staff in the Water Protection Program's Monitoring and Assessment Unit will use data that indicates potential impairment to determine where additional studies are needed. Department staff with the Environmental Services Program's Aquatic Bioassessment Unit will be used to conduct studies requested by the WPP.*

2. Identify appropriate EDU. *The Ecological Drainage Unit in which the test stream is located will be identified so that applicable biological criteria can be used to score macroinvertebrate data collected by Department biologists.*

3. Determine five variable VST of test stream segments (1st digit = temperature; 2nd digit = size; 3rd digit = flow; 4th digit = geology; and 5th digit = relative gradient). *This five-digit VST code provides a description of the test stream for later use in selecting appropriate candidate reference streams that are similar to the test stream (giving temperature, size, and flow the highest importance).*

4. Filter all stream segments within the same EDU for the relevant five variable VSTs (1st and 2nd digits especially critical for small streams). *The five VST features of the test stream will be determined by checking the "AQUATIC.STRM_SEGMENTS" layer in GIS software (e.g., ArcMap). This layer has an associated Attribute Table that has, among many other features, the five-digit VST code for classified Missouri streams. During the filtering process, the five-digit code (listed as "VST_5VAR" in the Attribute Table) of the test stream is chosen in an ArcMap tool called "Select by Attributes." The five-digit code of the test stream is entered into this ArcMap tool, which can then be used to list only streams with the same five VST variables while excluding (i.e., "filtering out") all other streams with different variables.*

5. Filter all potential VST stream segments for stressors against available GIS layers (e.g., point sources, landfills, CAFOs, lakes, reservoirs, mining, etc.). *A GIS layer that includes the stream segments selected in Step 4 will be created. The proximity of these selected stream layers will be evaluated relative to stressor layers cataloged in GIS using filtering steps similar to those described above. Stream segments with stressors having documented impacts will be eliminated from further consideration. The presence of a single potential stressor will not automatically lead to a stream reach being rejected; rather, the aggregate of potential stressors in a watershed will be evaluated.*

6. Filter all potential VST stream segments against historical reports and databases. *Past accounts of occurrences that may result in a stream failing to meet the "best available, least impaired" criteria will be evaluated. These incidents may include events such as fish kills, combined sewer overflows, or past environmental emergencies (e.g., releases of toxic substances). Exceptions can be made when the cause of the incident no longer exists and there are no lingering effects. In contrast, historical reports may also include studies by other biologists that support the use of a stream segment as a candidate reference stream.*

7. Calculate land use categories of candidate reference streams (e.g., percentage of forest, grassland, impervious surface, etc.) in GIS mapping software using available land cover datasets (Sources of land use data that are currently used are NLCD 2011 and MoRAP 2005). Candidate reference streams with the same or similar Aquatic Ecological System (AES) type as the test stream (within the EDU) will be given preference throughout the selection process. In addition, candidate reference streams should also be chosen from candidate reference stream watersheds whose land use composition is representative of test stream's AES, and generally representative of EDU land uses. Candidate reference stream watersheds will be excluded if impervious area covers greater than 10 percent of the watershed area *(Center for Watershed Protection, 2003).*

8. Develop candidate stream list with coordinates for field verification.

9. Field verify candidate list for actual use (e.g., animal grazing, in-stream habitat, riparian habitat), migration barriers (e.g., culverts, low water bridge crossings) representativeness, gravel mining, and other obvious human stressors. *Biologists can make additional fine-scale adjustments to the list of candidate streams by visiting sites in person. Certain features visible on-site may have been missed with GIS and other computer based filtering. Stream flow must be field verified to be similar to test streams.*

10. *Of the sites remaining after field verification and elimination, at least five of the top ranked candidate sites will be subjected to additional evaluation outlined below.*

> *For steps 4-9: These steps occur at the EDU level identified in step 2. These steps look at all streams within the identified EDU including those in the same (AES) Type as the test stream. Streams in the same AES Type as the test stream (within the identified EDU) will be given preference and be selected to go through the remaining steps (11-13) below.*

11. Collect chemical, biological, habitat, and possibly sediment field data. *Collection of physical samples is the ultimate manner in which the quality of a stream is judged. Although factors evaluated in the previous steps are good indicators of whether a stream is of reference quality, it is the evaluation of chemical, physical and biological attributes in relation to other candidate reference streams that is the final determinant. If chemical sampling documents an exceedance of water quality standards, the candidate reference stream will be eliminated from consideration.*

12. After multiple sampling events evaluate recent field data against available historical chemical, physical, biological, and land use data from each corresponding candidate reference stream. *Aquatic systems are subject to fluctuation due to weather, stream flow, and other climatic conditions. Land use in the watershed of a candidate reference also can change over time. It is therefore important to compare recent data to available historical data to evaluate if watershed conditions have changed over time. If this*

evaluation indicates that the candidate reference stream conditions are similar to or have improved relative to historical conditions, they will be retained. If historical data are not available to make the comparisons, the candidate reference streams will be retained.

13. If field data are satisfactory, retain candidate reference stream label in database. *Reference streams and candidate reference streams are labeled as such in a database maintained by the Department's Aquatic Bioassessment Unit in Jefferson City, Missouri.*

Fish Community Data

The Department utilizes fish community data to determine if aquatic life use is supported in certain types of Missouri streams. When properly evaluated, fish communities serve as important indicators of stream health. In Missouri, fish communities are surveyed by MDC. Each year, MDC selects an aquatic subregion from which randomly selected 2nd to 5th order sized streams are surveyed. Fish sampling follows procedures described in the document *Resource Assessment and Monitoring Program: Standard Operational Procedures--Fish Sampling* (Fischer & Combes 2011). Numeric biocriteria for fish are represented by the fIBI. Development of the fIBI is described in the document *Biological Criteria for Stream Fish Communities of Missouri* (Doisy *et al.* 2008).

The fIBI is a multi-metric index made up of nine individual metrics, which include:

- Number (No.) of native individuals;
- No. of native darter species;
- No. of native benthic species;
- No. of native water column species;
- No. of native minnow species;
- No. of all native lithophilic species;
- Percentage of native insectivore cyprinid individuals;
- Percentage of native sunfish individuals; and,
- Percentage of the three top dominant species.

Values for each metric, as directly calculated from the fish community sample, are converted to unitless scores of one, three, or five according to criteria in Doisy *et al.* (2008). The fIBI is then calculated by summing these unitless values for a total possible score of 45. Doisy *et al.* (2008) established an impairment threshold of 36 (where the 25th percentile of reference sites represent a score of 37), with values equal to or greater than 36 representing unimpaired communities and values less than 36 representing impaired communities. For more information regarding fIBI scoring, see Doisy *et al.* (2008).

Based on consultation between the Department and MDC, a fIBI impairment threshold value of 36 was used as the numeric biocriterion translator for making attainment decisions for aquatic life (Appendix C). However, because the work of Doisy *et al.* (2008) focused on streams 3rd to 5th order in size and fIBI was only validated for streams in the Ozark

ecoregion (not for streams in the Central Plains or Mississippi Alluvial Basin), fIBI may only be applied when assessing streams 3rd to 5th order in size from the Ozark ecoregion. Assessment procedures are outlined below.

Full Attainment

- For seven or fewer samples collected using MDC RAM fish community protocols, 75 percent of fIBI scores must be 36 or greater. Fauna achieving these scores are considered very similar to Ozark reference streams.
- For eight or more samples, the percentage scoring 36 or greater must be statistically similar to representative reference or control streams. To determine statistical similarity, a binomial probability Type I error rate (0.1) is calculated based on the null hypothesis that the test stream would have the same percentage (75 percent) of fIBI scores greater than 36 as reference streams. If the Type I error rate is more than the significance level α=0.1, the fish community is rated as unimpaired.

Non-Attainment

- For seven or fewer samples collected using MDC RAM fish community protocols, 75 percent of the fIBI scores must be lower than 36. Fauna achieving these scores are considered substantially different from regional reference streams.
- For eight or more samples, the percentage scoring 36 or less must be statistically dissimilar to representative reference or control streams. To determine statistical dissimilarity, a binomial probability Type I error rate is calculated based on the null hypothesis that the test stream would have the same percentage (75 percent) of fIBI scores greater than 36 as reference streams. If the Type I error rate is less than 0.1, the null hypothesis is rejected and the fish community is rated as impaired.

Data will be judged inconclusive when outcomes do not meet requirements for decisions of full or non-attainment.

With the exception of two subtle differences, use of the binomial probability for fish community samples will follow the example provided for macroinvertebrate samples in the previous section. First, instead of test stream samples being compared to reference streams of the same EDU, they will only be compared to reference streams from the Ozark ecoregion. Secondly, the probability of success used in the binomial distribution equation will always be set to 0.70 (see Appendix D for details).

Although 1st and 2nd order stream data will not be used to judge a stream as impaired for Section 303(d) purposes, the Department may use the above assessment procedures to judge 1st and 2nd order streams as unimpaired. Moreover, should samples contain fIBI scores less than 29 (Doisy *et al*. 2008), the Department may judge the stream as "suspected of impairment" using the above procedures.

Considerations for the Influence of Habitat Quality and Sample Representativeness

Low fIBI scores that are substantially different than reference streams could be the result of problems with water quality, habitat, or both. When low fIBI scores are determined, it is necessary to review additional information to differentiate between an impairment caused by water quality and one caused by habitat. Fish community sample collection is accompanied by a survey of physical habitat from the sampled reach. MDC sampling protocol for stream habitat follows procedures provided by Peck *et al.* (2006). With MDC guidance, the Department utilizes this habitat data and other available information to assure that a fish-based assessment of aquatic life attainment is only the result of water quality, and that an impairment resulting from poor or inadequate habitat is categorized as such. This section describes the procedures used to assure low fIBI scores are the result of water quality problems and not habitat degradation.

The information below outlines the Department's provisional method to identify unrepresentative samples and low fIBI scores of questionable habitat condition, and to ensure resulting fIBI scores are not used for Section 303(d) listing.

a) Following recommendations from the biocriteria workgroup, the Department will consult MDC about the habitat condition of particular streams when assessing low fIBI scores.

b) Samples may be considered for Section 303(d) listing ONLY if they were collected in the Ozark ecoregion, and, based upon best professional judgment from MDC staff, the samples were collected during normal representative conditions. Samples collected from the Central Plains and Mississippi Alluvial Basin cannot be appropriately evaluated and are excluded from Section 303(d) listing.

c) Only samples from streams 3rd to 5th order in size may be considered for Section 303(d) listing. Samples from 1st or 2nd order streams are excluded from Section 303(d) consideration; however, they may still be placed into Categories 2B and 3B if impairment is suspected, or into Categories 1, 2A, or 3A if sample scores indicate a stream is unimpaired. Samples from lower stream orders are surveyed under a different RAM Program protocol than 3rd to 5th order streams.

d) Samples that are ineligible for Section 303(d) listing include those collected from or in close proximity to losing streams, as defined by the Missouri Geological Survey. Additionally, ineligible samples may include those collected from streams with natural flow issues (such as streams reduced to predominately subsurface flow) that prevent good fIBI scores from being obtained, as determined through best professional judgment of MDC staff.

e) Fish IBI scores must be accompanied by a QCPH1 habitat index score. After analyzing meaningful habitat metrics and identifying samples where habitat

metrics seemed to indicate potential habitat concerns, MDC developed a provisional index named QCPH1. The QCPH1 comprises six sub-metrics indicative of substrate quality, channel disturbance, channel volume, channel spatial complexity, fish cover, as well as tractive force and velocity. QCPH1 values less than 0.39 indicate poor habitat, and values greater than 0.39 suggest adequate habitat.

The QCPH1 index is calculated as follows:

QCPH1 = ((Substrate Quality * Channel Disturbance * Channel Volume * Channel Spatial Complexity * Fish Cover * Tractive Force & Velocity)$^{1/6}$)

where sub-metrics are determined by:

Substrate Quality = [(embeddedness + small particles)/2] * [(filamentous algae + aquatic macrophyte)/2] * bedrock & hardpan

Channel Disturbance = concrete * riprap * inlet/outlet pipes * relative bed stability * residual pool observed to expected ratio

Channel Volume = [(dry substrate + width depth product + residual pool + wetted width)/4]

Channel Spatial Complexity = (coefficient of variation of mean depth + coefficient of variation of mean wetted width + fish cover variety)/3

Fish Cover = [(all natural fish cover + ((brush & overhanging vegetation + boulders + undercut bank + large woody debris)/4) + large types of fish cover)/3]

Tractive Force & Velocity = [(mean slope + depth * slope)/2]

Unimpaired fIBI samples (fIBI ≥36) with QCPH1 index scores below the 0.39 threshold value, or samples without a QCPH1 score altogether, are eliminated from consideration for Category 5 and, instead placed into Categories 2B or 3B should an impairment be suspected. Impaired fish communities (fIBI <36) with QCPH1 scores below 0.39 can be placed into Category 4C (non-discrete pollutant/habitat impairment). Impaired fish communities (fIBI <36) with adequate habitat scores (QCPH1 >0.39) can be placed into Category 5. Unimpaired fish communities (fIBI ≥36) and adequate habitat (QCPH1 >0.39) may be used to judge a stream as unimpaired.

Assessment of fish communities must be based on data coded Level 3 or 4 as described in Section II.C of this LMD. Data coded as Levels 3 and 4 represent environmental data with the greatest degree of assurance and indicate that assessments will include multiple samples from a single site or samples from multiple sites within a single reach.

Following the Department's provisional methodology, fish community samples available for assessment (using procedures in Appendix C & D) include only those from 3rd to 5th order Ozark Plateau streams with adequate habitat collected under normal representative conditions where there were no issues with inadequate flow or water volume.

Filamentous Algae Assessments

Department staff responsible for conducting percent coverage estimates for filamentous algae will make note of physical habitat components that might affect the presence and abundance of filamentous algae. Habitat notes will be included in the report. Potential habitat influences will then be considered during the assessment and when deciding which listing category is most appropriate. Control streams will be selected from within the same EDU, be of similar size (e.g. VST), and sampled in conjunction with the test stream. The Department's SOP for Estimation of Green Filamentous Algae (MDNR-ESP-020) is available upon request.

IV. Other Biological Data

On a case by case basis, the Department may use biological data other than MSCI or fib scores for assessing attainment of aquatic life. Other biological data may include information on single indicator aquatic species that are ecologically or recreationally important, or individual measures of community health that respond predictably to environmental stress. Measures of community health could be represented by aspects of structure, composition, individual health, and processes of the aquatic biota. Examples could include measures of density or diversity of aquatic organisms, replacement of pollution intolerant taxa, or even the presence of biochemical markers.

Acute or Chronic Toxicity Tests

If toxicity tests are to be used as part of the weight of evidence, then accompanying media (water or sediment) analyses must accompany toxicity test results (e.g., if metals are a concern, then metals concentrations in the sediment sample used for an acute toxicity test must accompany the toxicity test results; or, if PAHs are a concern, then PAHs concentrations and the Total Organic Carbon (TOC) concentration must accompany toxicity test results). The organism, its developmental stage used for the toxicity test, and the duration of the test must also accompany the results.

Other biological data should be collected under a well vetted study that is documented in a scientific report, a weight of evidence approach should be established, and the report should be referenced in the 303(d) listing worksheet. If other biological data is a critical component of the community and has been adversely affected by the presence of a pollutant or stressor, then such data would indicate a water body is impaired. The Department's use of other biological data is consistent with EPA's policy on independent

applicability for making attainment decisions, which is intended to protect against dismissing valuable information when diagnosing an impairment of aquatic life.

The use of other biological data is infrequent in water body assessments, but when available, it is usually assessed in combination with other information collected within the water body of interest. The Department will avoid using other biological data as the sole justification for a Section 303(d) listing; however, other biological data will be used as part of a weight of evidence analysis for making the most informed assessment decision.

V. Toxic Chemicals

1. Water

Standard acute or chronic bioassay procedures using freshwater aquatic fauna such as, but not limited to, water flea (*Ceriodaphnia dubia),* Fathead Minnows (*Pimephales promelas*), amphipod (*Hyalella azteca)*, or Rainbow Trout (*Oncorhynchus mykiss)*[19] will provide adequate evidence of toxicity for 303(d) listing purposes. Microtox® toxicity tests may be used to list a water as affected by "toxicity" only if data of another kind (freshwater toxicity tests, sediment or water chemistry, or biological sampling) also indicate water quality impairment.

For any given water, available data may occur throughout the system and/or be concentrated in certain areas. When the location of pollution sources are known, the Department reserves the right to assess data representative of impacted conditions separately from data representative of unimpacted conditions. Pollution sources include those that may occur at discrete points along a water body, or those that are more diffuse.

Chronic Toxicity Events

The parameters in WQS labeled as chronic criterion can be assessed in two ways:

1. Continuous Data Sondes
    - For data that has been collected consecutively over time, (e.g., a data sonde collecting information every 15 minutes for a two-week time period) the data will be used as is after QA/QC procedures.
2. Grab Samples
    - For samples that have not been collected consecutively, (e.g., a grab sample collected once a week) the hydrologic flow conditions of the stream or the closest USGS gage will be used to verify the sample was collected during stable flow conditions. Periods of unstable flow are not expected to be representative of averaging periods associated with chronic conditions. If the flow conditions were unstable, then the

[19] Reference 10 CSR 20-7.015(9)(L)(4)B for additional information

sample will not be assessed against the chronic criterion. If the flow conditions were stable, then the sample will be assessed against the chronic criterion. There are three categories of stable flow conditions: High, Medium, and Low.

i. High Stable Flow - greater than the 50th percentile exceedance flow, and less than 10 percent change in flow over a 48-hour period.

ii. Medium Stable Flow - between the 90th percentile exceedance flow and the 50th percentile exceedance flow, and less than 15 percent change in flow over a 48-hour period.

iii. Low Stable Flow - less than the 90th percentile exceedance flow or less than one cubic foot per second, and less than 20 percent change in flow over a 48-hour period.

- To additionally support judgment of the representativeness of conditions surrounding sampling events regarding flow and potential pollutant concentration duration; supplemental continuous data that may have an observed or documented relationship, such as specific conductance, may be incorporated into the assessment. Examples of how this data may be used could include linear regression, percent change over time, averages, etc. This data will only be used to support judgment of the representativeness of grab samples or the duration of the conditions observed. Supplemental data[20] alone (i.e., lacking any in-stream samples for the pollutant of concern) cannot be used to judge a waterbody as impaired.

2. Sediment

For toxic chemicals occurring in benthic sediments, a geometric mean will be calculated for specific toxins from an adequate number of samples (minimum of three samples). The calculated geometric mean will then be compared to the corresponding Probable Effect Concentration (PEC) given by MacDonald *et al.* (2000). The PEC is the level of a pollutant above which harmful effects on the aquatic community are likely to be observed. Refer to MacDonald *et al.* (2000) for the estimated accuracy of individual PECs to predict toxicity. For all metals listed in MacDonald *et al.* (2000) except arsenic (specifically cadmium, chromium, copper, lead, nickel, and zinc), pollutant geometric means will be compared to 150 percent of the recommended PEC values. These comparisons should meet confidence requirements applied elsewhere in this LMD. When multiple metal contaminants occur in sediment, toxicity may occur even though the level of each individual pollutant does not reach toxic levels. The method of estimating the synergistic effects of multiple metals in sediments is described below.

[20] Supplemental data in this context refers only to parameters that do not have applicable numeric criteria listed in 10 CSR 20.7.031. However, these parameters may represent indirect measurement of, or have an observed relationship to parameters that do have numeric criteria listed in 10 CSR 20.7.031.

The sediment PECs given by MacDonald *et al.* (2000) are based on additional data assumptions. Those assumptions include a 1 percent TOC content and that the sample has been sieved to less than 2 mm.

The Department uses 150 percent of the PEC values to account for variability in our assessment of sediment toxicity. See the ***Equilibrium Partitioning Sediment Benchmark*** section further below for more information on TOC and sulfide considerations for metals toxicity in sediment.

For the sample sieving assumption, the Department will use non-sieved (bulk) sediment concentrations for screening level data (Data Code One). Current impairments that have used bulk sediment data as evidence for impairment will remain on the 303(d) list until sieved data can be collected to show either that the water body should remain on the list or that the sieved concentrations are below the 150 percent PEC values. Data that has been sieved to less than 2 mm or smaller will be used for comparison to the 150 percent PEC values.

The Meaning of the Sediment Quotient and How to Calculate It

Although sediment criteria in the form of PECs are given for several individual contaminants, it is recognized that when multiple contaminants occur in sediment, toxicity may occur even though the level of each individual pollutant does not reach toxic levels. The MacDonald *et al.* (2000) method for estimating the synergistic effects of multiple pollutants in sediment utilizes the calculation of a Probable Effects Concentration Quotient (PECQ). PECQs greater than 0.75 will be judged as toxic.

This calculation is made by dividing the pollutant concentration in the sample by the PEC value for that pollutant. When multiple samples are available, the geometric mean (as calculated for each specific pollutant) will be placed in the numerator position for each pollutant included in the equation. To ensure a complete picture of the synergistic effects of metals, the seven metals required to be included in the PECQ are: arsenic, cadmium, chromium, copper, lead, nickel, and zinc[21].

Example - Three sediment samples result in the following geometric means (values are in mg/kg):

Arsenic 2.5, Cadmium 4.5, Chromium 135.4, Copper 85.6, Lead 100.0, Nickel 65.8, and Zinc 260.0.

The PEC values for these seven pollutants in respective order are:

33, 4.98, 111, 149, 128, 48.6 and 459 mg/kg.

[21] Ingersoll et. al 2000 Prediction of sediment toxicity using consensus-based freshwater sediment quality guidelines https://www.cerc.usgs.gov/pubs/center/pdfdocs/91126.pdf

PECQ = [(2.5/33) + (4.5/4.98) + (135.4/111) + (85.6/149) + (100/128) + (65.8/48.6) + (260/459)]/7 = 0. 782

Using PECQ to Judge Metals Toxicity

Based on research by MacDonald *et al.* (2000), 83 percent of sediment samples with a PECQ less than 0.5 were non-toxic; while 85 percent of sediment samples with a PECQ greater than 0.5 were toxic. Therefore, to accurately assess the synergistic effects of sediment contaminants on aquatic life, the Department will judge a PECQ greater than 0.75 as toxic**.**

Using Total PAHs to Judge Toxicity

PAHs are organic compounds containing only carbon and hydrogen that form aromatic rings (cyclic molecular shapes). PAHs in the environment can be of natural origin, such as from coal and oil deposits, or man-made (anthropogenic) from the use and breakdown of hydrocarbon compounds. There are three different sources of hydrocarbon compounds: plants (phytogenic), petroleum (petrogenic), and the combustion of petroleum, wood, coal, etc. (pyrogenic). In streams, the most common sources of PAHs are from anthropogenic sources such as sealants (coal tar) and other treatments of roads, driveways, and parking lots.

Mount *et al.* (2003) indicates that individual PAH guidelines (PECs) are based on the samples also having an elevated presence of other PAHs. This potentially overestimates the actual toxicity of an individual PAH PEC value. A *Total* PAH guideline (e.g., PEC) reduces variability and provides a better representation of toxicity when compared to individual PAH PECs.

Based on research by MacDonald *et al.* (2000), 81.5 percent of sediment samples with a Total PAH value less than 22.8 mg/kg were non-toxic; while 100 percent of sediment samples with a Total PAH value greater than 22.8 mg/kg were toxic. Therefore, to accurately assess the toxicity to aquatic life of total PAHs in sediment, the Department will judge Total PAH values greater than 150 percent of the PEC value (34.2 mg/kg) as toxic.

What compounds are considered in calculating Total PAHs and how will they be compared to the 150 percent PEC value?

To calculate Total PAHs for a sample, Mount *et al.* (2003) recommends following the EPA Environmental Monitoring Assessment Program's definition of Total PAHs. This definition includes 34 PAH compounds; 18 parent PAHs and 16 alkylated PAHs (see Table 3 for a list of these compounds). Mount *et al.* (2003) indicates that using less than the 34 PAH compounds can underestimate the toxicity of PAHs in sediment. TOC has the potential to affect the bioavailability of PAHs. Organic carbon can provide a binding phase for PAHs, but the extent of that binding capacity is unknown. Through the weight of evidence approach (see Section D. II.), the Department will consider the effects of TOC on a case by case basis.

Only 14 to18 of the 34 PAH compounds are commonly requested for analysis. Therefore, the process to judge toxicity due to Total PAHs is as follows:

- To evaluate Total PAH compounds: First sum all individual PAH compounds per sample to calculate the Total PAHs for that sample. Next take the geometric mean of all of the individual sample totals. The resulting geometric mean can then be compared to the PEC or 150 percent PEC.
- When samples are analyzed for fewer than the 34 PAH compounds:
  - If the geometric mean for more than one sample is greater than the 150 percent PEC, then the samples will be judged as toxic.
  - If the geometric mean for more than one sample is greater than the 100 percent but less than 150 percent of the PEC, then the samples will be judged as inconclusive.
  - If the geometric mean for more than one sample is less than the 100 percent PEC, then the values will be judged as non-toxic.
- When samples are analyzed for all 34 PAH compounds:
  - If the geometric mean for more than one sample is greater than the 150 percent PEC, then the samples will be judged as toxic.
  - If the sum of the geometric means for more than one sample is less than the 150% PEC, then the samples will be judged as non-toxic.

Table 3. List of 34 polycyclic aromatic hydrocarbon (PAH) compounds that are considered for the calculation of Total PAHs.

Parent PAHs (18)	Alkylated PAHs (16)
Acenaphthene	C1-Benzanthracene/chrysenes
Acenphthylene	C1-Fluorenes
Anthracene*	C1-Naphthalenes
Benz(a)anthracene*	C1-Phenanthrene/anthracenes
Benzo(a)pyrene*	C1-Pyrene/fluoranthenes
Benzo(b)fluoranthene	C2-Benzanthracene/chrysenes
Benzo(e)pyrene	C2-Fluorenes
Benzo(g,h,i)perylene	C2-Naphthalenes
Benzo(k)fluoranthene	C2-Phenanthrene/anthracenes
Chrysene*	C3-Benzanthracene/chrysenes
Dibenz(a,h)anthracene	C3-Fluorenes
Fluoranthene*	C3-Naphthalenes
Fluorene*	C3-Phenanthrene/anthracenes
Indeno(1,2,3-cd)pyrene	C4-Benzanthracene/chrysenes
Naphthalene*	C4-Naphthalenes
Perylene	C4-Phenanthracene/anthracenes
Phenanthrene*	
Pyrene*	

**Listed in Table 3 of MacDonald et al. (2000)*

Equilibrium Partitioning Sediment Benchmark (ESB) Data

Another type of analysis for sediment metal toxicity is based on the EPA (2006) paper that discusses ESBs and their uses. The Department does not currently collect ESB data but will consider ESB data collected by other entities under the weight of evidence approach. To be considered, the data must be accompanied by the name of the laboratory having completed the analysis, along with a copy of their laboratory procedures and QC documentation. Sieved sediment samples will be judged as toxic for metals in sediment if the sum of the simultaneously extracted metals minus acid volatile sulfides, divided by the fractional organic carbon [(ΣSEM-AVS)/FOC] is greater than 3000. If additional sieved sediment samples also show toxicity for a particular metal(s), then that particular metal(s) will be identified as the cause for toxicity.

Pictorial representations (flow charts) for how these different sediment toxicity procedures could be used in the weight of evidence analysis are displayed in Appendix E.

VI. Duration of Assessment Period

Except where the assessment period is specifically noted in Appendix B, the time period during which data will be used in making the assessments will be determined by data age and data code considerations, as well as representativeness considerations such as those described in footnote 14.

VII. Assessment of Tier Three Waters

Waters given Tier Three Protection by the antidegradation rule at 10 CSR 20-7.031(3) shall be considered impaired if data indicate water quality has been reduced in comparison to its historical quality. Historical water quality is determined from past data that best describes a water's quality following promulgation of the antidegradation rule and at the time the water was given Tier Three Protection.

Historical data gathered at the time a water body was given Tier Three Protection will be used if available. Because historical data may be limited, the historical quality of waters may be determined by comparing data from the assessed segment with data from a "representative" segment. A representative segment is a body or stretch of water that best reflects the conditions that probably existed at the time the antidegradation rule first applied to the waters being assessed. Examples of possible representative data include data from stream segments upstream of assessed segments that receive discharges, and data from other water bodies in the same ecoregion that have similar watershed and landscape characteristics. These representative stream segments would also be characterized as having the quality and quantity of receiving discharges similar to those of the historic discharges of the assessed segment. The assessment may also use data from the assessed segment gathered between the time the Tier Three Protection was initiated and the last known time in which

upstream discharges, runoff, and watershed conditions remained the same, provided that the data do not show any significant trends of declining water quality during that period.

The data used in the comparisons will be tested for normality and an appropriate statistical test will be applied. The null hypothesis for statistical analysis will be that water quality at the test segment and representative segment are the same. This will be a one-tailed test (the test will consider only the possibility that the assessed segment has poorer water quality) with the alpha level (α) of 0.1. This means that the test must show greater than a 90 percent probability that the assessed segment has poorer water quality than the representative segment before the assessed segment can be listed as impaired.

VIII. Other Types of Information

1. *Observation and evaluation of waters for noncompliance with state narrative water quality criteria*. Missouri's general (narrative) water quality criteria, as described in 10 CSR 20-7.031 Section (4), may be used to evaluate waters when a quantitative (numeric) value cannot be applied to the pollutant. These narrative criteria apply to both classified and unclassified waters and prohibit the following in waters of the state:
    a. Waters shall be free from substances in sufficient amounts to cause the formation of putrescent, unsightly, or harmful bottom deposits or prevent full maintenance of beneficial uses;
    b. Waters shall be free from oil, scum, and floating debris in sufficient amounts to be unsightly or prevent full maintenance of beneficial uses;
    c. Waters shall be free from substances in sufficient amounts to cause unsightly color or turbidity, offensive odor, or prevent full maintenance of beneficial uses;
    d. Waters shall be free from substances or conditions in sufficient amounts to result in toxicity to human, animal, or aquatic life;
    e. Waters shall maintain a level of water quality at their confluences to downstream waters that provides for the attainment and maintenance of the WQS of those downstream waters, including waters of another state;
    f. There shall be no significant human health hazard from incidental contact with the water;
    g. There shall be no acute toxicity to livestock or wildlife watering;
    h. Waters shall be free from physical, chemical, or hydrologic changes that would impair the natural biological community; and
    i. Waters shall be free from used tires, car bodies, appliances, demolition debris, used vehicles or equipment, and solid waste as defined in Missouri's Solid Waste Law, section 260.200, RSMo, except where the use of such materials is specifically permitted pursuant to sections 260.200–260.247, RSMo.
2. *Evaluation of aquatic habitat to further inform assessment decisions.* Habitat assessment protocols for wadeable streams have been established and are conducted in conjunction

with sampling aquatic macroinvertebrates and fish. Methods for evaluating aquatic macroinvertebrate and fish community data include assessment procedures that account for the presence or absence of representative habitat quality. The Department will not use habitat data alone for assessment purposes.

E. Other 303(d) Listing Considerations

- *Adding to the Existing List or Expanding the Scope of Impairment to* a *Previously Listed Water Body:*

  Following the guidelines set forth in this LMD, the listed portion of an impaired water body may be increased, or one or more new pollutants may be added to a listing, based on more recent monitoring data. Waters not previously listed may also be added to the list under the guidelines set forth in this LMD.

- *Deleting from the Existing List or Decreasing the Scope of Impairment to* a *Previously Listed Water Body:*

  Following the guidelines set forth in this LMD, the listed portion of an impaired water body may be decreased, or one or more pollutants may be removed from the listing, based on more recent monitoring data (see Appendix D). Waters may also be completely removed from the list for several reasons;[22] the most common being that the water is attaining WQS, or that the water has an EPA approved TMDL or permit-in-lieu of a TMDL.

- *Assessment Units and Length of Impaired Segments:*

  In many circumstances, the Department will use the water body ID (WBID) listed in the MUDD as the "Assessment Unit" (AU) reported to EPA. However, in circumstances where the Department is able to more accurately assess attainment status for specific portions of a reach that are smaller than the WBID, the Department will create refined AUs within the geospatial extent of the WBID listed in the MUDD, while maintaining a link to the WBID. This subsegmentation of a WBID into smaller AUs is for assessment purposes only and does not change the underlying water quality standards associated with the overall WBID. The designated uses, criteria, and antidegradation provisions applicable to the refined AU will continue to be based on Missouri WQS using the linked WBID in the MUDD. When specifying AUs, the Department will provide justification in the corresponding assessment worksheets when WBIDs are divided into smaller AUs. The Department will welcome discussion of such proposed divisions during the public notice period of the 303(d) list. The measured impairment length of a 303(d) listing will be based on either the WBID length from the MUDD, or the refined segment length of the refined AU as appropriate.

[22] See, "Guidance for 2006 Assessment, Listing and Reporting Requirements Pursuant to Sections 303(d), 305(b) and 314 of the Clean Water Act". USEPA, Office of Water, Washington DC.

The process for refined AUs will follow EPA guidance.[23] Specifically the guidance states the following:

"*In this guidance, the term "segment" is synonymous with the term "assessment unit" (AU) used in previous IR Guidance. Fundamental to this accounting is the use of a consistent and rational segmentation and geo-referencing approach for all segments including rivers, streams, lakes, wetlands, estuaries, and coastal waters.*
EPA recommends that states consider using the National Hydrography Dataset (NHD) coding scheme to georeference their segments, where segments may comprise part of an NHD reach, an individual NHD reach, or a collection of NHD reaches or parts of reaches. Alternatively, if a state has already developed a comparable Geographic Information System (GIS) framework, EPA requests that states provide any relevant information necessary to allow consistent georeferencing. Additionally, this information should be included in the state's ADB submission. States generally partition waters to represent homogeneity in physical, biological or chemical conditions. This segmentation may reflect an a priori knowledge of factors such as flow, channel morphology, substrate, riparian condition, adjoining land uses, confluence with other waterbodies, and potential sources of pollutant loadings (both point and nonpoint). While there is no single default dimension for a segment size, states have utilized these or similar principles when they defined the segments used in their water quality standards. Other factors may include the following:

- *The expected natural variability of the measured criteria associated with the WQSs.*
- *The type of water (e.g., a small stream, a wide river, a tidal and stratified estuary, and coastal shoreline).*
- *Time of travel of a parcel of water in the waterbody or segment or the magnitude of any tidal excursions.*
- *The amount of and type of data and information necessary to provide a reasonably accurate characterization of the criteria (or core indicators) associated with the designated uses in the segment or waterbody.*
- *Any expected changes in significant influences in the watershed (land use, point or nonpoint sources of pollutants).*
- *Any site-specific concerns such as patchy or unique habitat distribution patterns or biological population distributions."*

<u>General precautions for the Department's refinement of AUs:</u>

- AUs will be maintained in a geospatial dataset, which uses the MUDD as a reference point.
- Generally, the WBID will function as the default AU, unless or until data and information is collected such that the Department can further refine the scope of water quality changes or impacts
- The Department's process for refining AUs will be updated as needed to make sure the Department is consistently defining AUs and to incorporate public feedback on the process

[23] EPA 2006 Integrated Report Guidance: https://www.epa.gov/sites/default/files/2015-10/documents/2006irg-report.pdf

- The Department will utilize geospatial tools to ensure consistency in the process
- AU names and IDs will follow a naming convention that allows for relatively easy identification and transparency
- The Department will "err on the side of caution" defaulting to larger AUs. Meaning if there is uncertainty, the Department will not split an existing AU until further information is collected that sufficiently justifies splitting the AU
- The minimum size of an AU will be limited to one single NHD reach. Maximum size is limited by the geospatial extent of the WBID in the MUDD
- AU attributes must maintain a link to the applicable WBID in the MUDD. The WBID in the MUDD determines which designated uses and criteria apply within the AU
- One WBID may be split into one or more AUs, unless that WBID is comprised of only a single NHD reach or only a portion of a NHD reach.
- AUs cannot span multiple WBIDs
- At this time individual lakes or reservoirs will not be split into AUs representing sections or arms. However, individual "Presumed Use Lakes" may be segmented out from of the HUC 8 based WBIDs.
- For each listing cycle, as part of the public notice for the 303(d) list, the Department will provide a summary of any new or changed AUs, with a short explanation. This summary will include a cross-reference to both the WQS segment (WBID) as well as any prior AU. Additionally, all AUs will need to be entered and tracked in EPA's ATTAINS database.

F. Prioritization of Waters for TMDL Development

Section 303(d) of the CWA and federal regulation 40 CFR 130.7(b)(4) requires states to submit a priority ranking of waters requiring TMDLs. The Department will prioritize development of TMDLs based the prioritization framework[24] which includes variables such as:

- social impact/public interest and risk to public health;
- toxicity to aquatic life
- nutrient reduction
- complexity and cost (including consideration of budget constraints);
- availability of data of sufficient quality and quantity for TMDL modeling;
- court orders, consent decrees, or other formal agreements;
- source of impairments;
- existence of appropriate numeric quality criteria;
- implementation potential and amenability of the problem to treatment;
- planning goals established in Missouri's Nonpoint Source Management Plan; and
- Integrated Planning efforts by municipalities and other entities.

[24] https://dnr.mo.gov/document-search/prioritization-framework-development-total-maximum-daily-loads

The Department's TMDL schedule and prioritization are included on the 303(d) list. The TMDL unit in the Water Protection Program develops the TMDL schedule and framework. More information is available from the following website: https://dnr.mo.gov/water/what-were-doing/water-planning/quality-standards-impaired-waters-total-maximum-daily-loads/tmdls.

G. Resolution of Interstate/International Disagreements

The Department will review the draft 303(d) lists of all states with which it shares a border or other interstate waters (e.g., Missouri River, Mississippi River, Des Moines River, St. Francis River). Where the listing in another state is different than the one in Missouri for the same water body, the Department will request the data and listing justification from the other state. The data will be reviewed following the evaluation guidelines in this LMD. The draft Missouri 303(d) list may be subject to change pending the results of any such evaluation.

H. Statistical Considerations

The most recent EPA guidance on the use of statistics in the 303(d) LMD is given in Appendix A. Within this guidance are three major recommendations regarding statistics:

- Provide a description of analytical tools used by the state under various circumstances;
- Explain the various circumstances under which the burden-of-proof is placed on proving the water is impaired and when it is placed on proving the water is unimpaired (applicable to hypothesis testing); and
- Explain the level of statistical significance (α) used under various circumstances.

- Description of Analytical Tools

  Appendix D describes the analytical tools the Department will use to determine whether a water body is impaired and whether or when a listed water body is no longer impaired.

- Rationale for the Burden-of-Proof

  Hypothesis testing is a common statistical analysis in which an assumed observation, or alternate hypothesis, is tested by comparison with a null hypothesis. The procedure involves first stating a testable observation (alternate hypothesis), such as, "The most frequently seen clothing color at a St. Louis Cardinals game is red," and then the opposite, which becomes the null hypothesis ("Red is not the most frequently seen clothing color at a St. Louis Cardinals game."). A statistical test is then applied to the data (e.g., a sample of the predominant clothing color worn by 200 fans at a St. Louis Cardinals game on July 12, 2019) and based on the result of that analysis, one of the two hypotheses is chosen as correct.

  In hypothesis testing, the burden-of-proof is always on the alternate hypothesis. In other words, the data must be especially convincing to make us reject the null hypothesis and accept the alternate hypothesis as being true. How convincing the data must be is stated as the "significance level" of the test. A significance level of $\alpha = 0.10$ means that there must be at least

a 90 percent probability that the alternate hypothesis is true before we can accept it and reject the null hypothesis.

For analysis of a specific kind of data, either the test significance level or the statement of null and alternative hypotheses, or both, can be varied to achieve the desired level of statistical rigor. The Department has chosen to maintain a consistent set of null and alternate hypotheses for all statistical tests. The null hypothesis will be that the water body in question is unimpaired, and the alternate hypothesis will be that it is impaired. The Department's desired level of statistical rigor will be accomplished by varying the test significance level. For determining impairment (Appendix D), significance levels are set at either $\alpha = 0.1$ or $\alpha = 0.4$, meaning the data must show at minimum a 90 percent or 60 percent probability, respectively, that the water body is impaired. However, if the Department retained these same significance levels to determine when an impaired water body has been restored to an unimpaired status (Appendix D), some undesirable results can occur.

For example, when using a significance level of $\alpha = 0.1$ for determining both impairment and non-impairment, if sample data indicate a stream had a 92 percent probability of being impaired, it would be rated as impaired. If subsequent data were collected and added to the database, and the data now showed the water had an 88 percent probability of being impaired, it would be rated as unimpaired. Judging a water body with only a 12 percent probability of being unimpaired as unimpaired is clearly a poor decision. To correct this problem, the Department will use a test significance level of $\alpha = 0.4$ for some analytes and $\alpha = 0.6$ for others. This will increase Department confidence in determining WQS compliance to 40 percent and 60 percent, respectively, under the worst-case conditions.

- Level of Significance Used in Tests

Significance levels are chosen with two concerns in mind. The first concern involves matching decision error rates with the severity of the consequences of committing a decision error. The second concern addresses the need to balance, within practicality, Type I (the error of rejecting a null hypothesis when it is actually true) and Type II (the error of not rejecting a null hypothesis when the alternative hypothesis is true) error rates. For relatively small sample numbers, the disparity between Type I and Type II errors can be large. Tables 4 and 5 display calculated error rates using the binomial distribution for two very similar situations. Type I and Type II error rates are based on a stream with a 10 percent and a 15 percent WQS exceedance rate, respectively. Note that when sample size remains the same (Table 4), Type II error rates increase as Type I error rates decrease. Also note that for a given Type I error rate, the Type II error rate declines as sample size increases (Table 5).

Table 4. Effects of Type I error rates on Type II error rates when sample sizes are equal. Type I and Type II error rates are based on a stream with a 10 percent and a 15 percent WQS exceedance rate, respectively.

Total No. of Samples	No. Samples Meeting Std.	Type I Error Rate	Type II Error Rate
18	17	0.850	0.479

18	16	0.550	0.719
18	15	0.266	0.897
18	14	0.098	0.958
18	13	0.028	0.988

Table 5. Effects of Type I error rates and sample size on Type II error rates. Type I and Type II error rates are based on a stream with a 10 percent and a 15 percent WQS exceedance rate, respectively.

Total No. of Samples	No. Samples Meeting Std.	Type I Error Rate	Type II Error Rate
6	5	0.469	0.953
11	9	0.303	0.930
18	15	0.266	0.897
25	21	0.236	0.836

- Use of the Binomial Probability Distribution for Interpretation of the 10 Percent Rule

  There are two options for assessing data for compliance with the 10 percent rule[25]. One is to simply calculate the percent of time the criterion value is not met, and to judge the water to be impaired if this value is greater than 10 percent. The second method is to use an evaluative procedure that can review the data and provide a probability statement regarding compliance with the 10 percent rule. Since the latter option allows assessment decisions relative to specific test significance levels and the former option does not, the latter option is preferred. The Department uses the binomial probability distribution and calculation of the Type I error rate as the evaluative procedure.

- Other Statistical Considerations

  Prior to calculating confidence limits, the normality of the dataset will be evaluated. If normality is improved by data transformation, the confidence limits will be calculated on the transformed data.

  Time of sample collection may be biased and interfere with an accurate measurement of the frequency of criterion exceedance. Data sets composed mainly or entirely of storm water data or data collected only during a season when water quality problems are expected could result in a biased estimate of the true exceedance frequency. In these cases, the Department may use methods to estimate the true annual frequency and display these calculations whenever they result in a change in the impairment status of a water body.

[25]Guidelines for preparation of the Comprehensive State Water Quality Assessments (305(b) Reports) and Electronic Updates (1997) Supplement Volume 2. Refer to page 80, section on conventionals (dissolved oxygen, pH, temperature). https://www.epa.gov/sites/default/files/2015-09/documents/guidelines_for_preparation_of_the_comprehensive_state_water_quality_assessments_305b_reports_and_electronic_updates_1997_supplement-volume2.pdf#page=80

For waters judged to be impaired based on biological data where data evaluation procedures are not specifically noted in Table 1, the statistical procedure used, test assumptions, and results will be reported.

- Examples of Statistical Procedures

Two Sample "t" Test for Color

Null Hypothesis: The amount of color is no greater in a test stream than in a control stream. As stated, this is a one-sided test, meaning that we are only interested in determining whether or not the color level in the test stream is greater than in a control stream. If the null hypothesis had been "the amount of color is different in the test and control streams," we would have been interested in determining if the amount of color was either less than or greater than the control stream, a two-sided test.

Significance Level: $\alpha = 0.10$

Data Set: Platinum-Cobalt color scale data for the test stream and a control stream. Samples were collected at each stream on the same date.

Test Stream (T)	70	45	35	45	60	60	80
Control Stream (C)	50	40	20	40	30	40	75
Difference (T-C)	20	5	15	5	30	20	5

Statistics for the Difference: Mean = 14.28, standard deviation = 9.76, n = 7
Calculated "t" value = (square root of n)(mean)/standard deviation = **3.86**
Tabular "t" value is taken from a table of the "t" distribution for 2 alpha (0.20) and n-1 degrees of freedom. Tabular "t" = **1.44**

Since the calculated "t" value is greater than tabular "t" value, we reject the null hypothesis and conclude that the amount of color in the test stream is greater than the control stream (i.e., the test stream is impaired by color).

Statistical Procedure for Mercury in Fish Tissue

Data Set (in µg/Kg): 130, 230, 450. Mean = 270, Standard Deviation = 163.7
If the 60% Lower Confidence Limit (LCL) Interval = the sample mean minus the quantity; and ((0.253)(163.7)/square root 3) = 23.9. Then, the 60 percent LCL Interval is 246.1 µg/Kg.

The criterion value is 300 µg/Kg. Since the 60 percent LCL Interval is less than the criterion value, the water is judged to be unimpaired by mercury in fish tissue, and the water body is placed in either Category 2B or 3B.

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Appendix A

Excerpt from *Guidance for 2006 Assessment, Listing and Reporting Requirements Pursuant to Sections 303(d), 305(b) and 314 of the Clean Water Act.* July 29, 2005. US EPA pp. 39-41.

The document can be read in its entirety from the US. EPA web site:
http://water.epa.gov/lawsregs/lawsguidance/cwa/tmdl/upload/2006irg-report.pdf

How should statistical approaches be used in attainment determinations?

The state's methodology should provide a rationale for any statistical interpretation of data for the purpose of making an assessment determination.

Description of statistical methods to be employed in various circumstances

The methodology should provide a clear explanation of which analytic tools the state uses and under which circumstances. EPA recommends that the methodology explain issues such as the selection of key sample statistics (arithmetic mean concentration, median concentration, or a percentile), null and alternative hypotheses, confidence intervals, and Type I and Type II error thresholds. The choice of a statistic tool should be based on the known or expected distribution of the concentration of the pollutant in the segment (e.g., normal or log normal) in both time and space.

Past EPA guidance (1997 305(b) and 2000 CALM) recommended making non-attainment decisions, for "conventional pollutants[26]" — TSS, pH, BOD, fecal coliform bacteria, and oil and grease — when more than "10% of measurements exceed the water quality criterion." (However, EPA guidance has not encouraged use of the "10% rule" with other pollutants, including toxics.) Use of this rule when addressing conventional pollutants, is appropriate if its application is consistent with the manner in which applicable WQC are expressed. An example of a WQC for which an assessment based on the ten percent rule would be appropriate is the EPA acute WQC for fecal coliform bacteria, applicable to protection of water contact recreational use. This 1976-issued WQC was expressed as, "...no more than ten percent of the samples exceeding 400 CFU per 100 ml, during a 30-day period." Here, the assessment methodology is clearly reflective of the WQC.

On the other hand, use of the ten percent rule for interpreting water quality data is usually not consistent with WQC expressed either as: 1) instantaneous maxima not to be surpassed at any time, or 2) average concentrations over specified times. In the case of "instantaneous maxima (or minima) never to occur" criteria use of the ten percent rule typically leads to the belief that segment conditions are equal or better than specified by the WQC, when they in fact are considerably worse. (That is, pollutant concentrations are above the criterion-concentration a far greater

[26] There are a variety of definitions for the term "conventional pollutants." Wherever this term is referred to in this guidance, it means "a pollutant other than a toxic pollutant."

proportion of the time than specified by the WQC.) Conversely, use of this decision rule in concert with WQC expressed as average concentrations over specific times can lead to concluding that segment conditions are worse than WQC, when in fact they are not.

If the state applies different decision rules for different types of pollutants (e.g., toxic, conventional, and non-conventional pollutants) and types of standards (e.g., acute vs. chronic criteria for aquatic life or human health), the state should provide a reasonable rationale supporting the choice of a particular statistical approach to each of its different sets of pollutants and types of standards.

1. *Elucidation of policy choices embedded in selection of particular statistical approaches and use of certain assumptions EPA strongly encourages states to highlight policy decisions implicit in the statistical analysis that they have chosen to employ in various circumstances. For example, if hypothesis testing is used, the state should make its decision-making rules transparent by explaining why it chose either "meeting WQS" or "not meeting WQS" as the null hypothesis (rebuttable presumption) as a general rule for all waters, a category of waters, or an individual segment. Starting with the assumption that a water is "healthy" when employing hypothesis testing means that a segment will be identified as impaired, and placed in Category 4 or 5, only if substantial amounts of credible evidence exist to refute that presumption. By contrast, making the null hypothesis "WQS not being met" shifts the burden of proof to those who believe the segment is, in fact, meeting WQS.*

Which "null hypothesis" a state selects could likely create contrasting incentives regarding support for additional ambient monitoring among different stakeholders. If the null hypothesis is "meeting standards," there were no previous data on the segment, and no additional existing and readily available data and information are collected, then the "null hypothesis" cannot be rejected, and the segment would not be placed in Category 4 or 5. In this situation, those concerned about possible adverse consequences of having a segment declared "impaired" might have little interest in collection of additional ambient data. Meanwhile, users of the segment would likely want to have the segment monitored, so they can be ensured that it is indeed capable of supporting the uses of concern. On the other hand, if the null hypothesis is changed to "segment not meeting WQS," then those that would prefer that a particular segment not be labeled "impaired" would probably want more data collected, in hopes of proving that the null hypothesis is not true.

Another key policy issue in hypothesis testing is what significance level to use in deciding whether to reject the null hypothesis. Picking a high level of significance for rejecting the null hypothesis means that great emphasis is being placed on avoiding a Type I error (rejecting the null hypothesis, when in fact, the null hypothesis is true). This means that if a 0.10 significance level is chosen, the state wants to keep the chance of making a Type I error at or below ten percent. Hence, if the chosen null hypothesis is "segment meeting

WQS," the state is trying to keep the chance of saying a segment is impaired – when in reality it is not – under ten percent.

An additional policy issue is the Type II errors (not rejecting the null hypothesis, when it should have been). The probability of Type II errors depends on several factors. One key factor is the number of samples available. With a fixed number of samples, as the probability of Type I error decreases, the probability of a Type II error increases. States would ideally collect enough samples so the chances of making Type I and Type II errors are simultaneously small. Unfortunately, resources needed to collect such numbers of samples are quite often not available.

The final example of a policy issue that a state should describe is the rationale for concentrating limited resources to support data collection and statistical analysis in segments where there are documented water quality problems or where the combination of nonpoint source loadings and point source discharges would indicate a strong potential for a water quality problem to exist.

EPA recommends that, when picking the decision rules and statistical methods to be utilized when interpreting data and information, states attempt to minimize the chances of making either of the two following errors:

- *Concluding the segment is impaired, when in fact it is not, and*
- *Deciding not to declare a segment impaired, when it is in fact impaired.*

States should specify in their methodology what significance level they have chosen to use, in various circumstances. The methodology would best describe in "plain English" the likelihood of deciding to list a segment that in reality is not impaired (Type I error if the null hypothesis is "segment not impaired"). Also, EPA encourages states to estimate, in their assessment databases, the probability of making a Type II error (not putting on the 303(d) list a segment that in fact fails to meet WQS), when: 1) commonly-available numbers of grab samples are available, and 2) the degree of variance in pollutant concentrations are at commonly encountered levels. For example, if an assessment is being performed with a WQC expressed as a 30-day average concentration of a certain pollutant, it would be useful to estimate the probability of a Type II error when the number of available samples over a 30 day period is equal to the average number of samples for that pollutant in segments state-wide, or in a given group of segments, assuming a degree of variance in levels of the pollutant often observed over typical 30 day periods.

Appendix B

METHODS FOR ASSESSING COMPLIANCE WITH WQS USED FOR 303(d) LISTING PURPOSES: NUMERIC CRITERIA THAT ARE INCLUDED IN STATE WQS (10 CSR 20-7.031)

DESIGNATED USES	DATA TYPE	DATA QUALITY CODE	COMPLIANCE WITH WQS[i]	Notes
Overall use protection (all designated uses)	No data. Evaluated based on similar land use/geology as stream with water quality data.	Not applicable	Given same rating as monitored stream with same land use and geology.	**Data Type Note:** This data type is used only for wide-scale assessments of aquatic biota and aquatic habitat for 305(b) Report purposes. This data type is not used in the development of the 303(d) list.
Any designated uses	No data available or where only effluent data is available. Results of dilution calculations or water quality modeling.	Not applicable	Where models or other dilution calculations indicate noncompliance with allowable pollutant levels and frequencies noted in this table, waters may be added to Category 3B and considered high priority for water quality monitoring.	
Protection of Aquatic Life	Dissolved oxygen, water temperature, pH, total dissolved gases, oil and grease	1-4	Full: No more than 10 percent of all samples exceed criterion. Non-Attainment: Requirements for full attainment not met. Requirements: A minimum sample size of 10 samples during the assessment period (see Section VI above).	**Compliance with WQS Note:** Some sampling periods are wholly or predominantly during the critical period of the year when criteria violations occur. Where the monitoring program presents good evidence of a demarcation between seasons where criteria exceedances occur and seasons when they do not, the 10 percent exceedance rate will be based on an annual estimate of the frequency of exceedance. Continuous (e.g., sonde) data with a quality rating of excellent or good will be used for assessments.
Protection of Aquatic Life	Chemical (toxins)	1-4	Full: No more than one acute toxic event in three years that results in a documented die-off of aquatic life such as fish, mussels, and crayfish (does not include die-offs due to natural origin). No more than one exceedance of acute or chronic criterion in the last three years for which data is available. Non-Attainment: Requirements for full attainment not met.	**Compliance with WQS Note:** For hardness-based metals with eight or fewer samples, the hardness value associated with the sample will be used to calculate the acute or chronic thresholds. For hardness-based metals with more than eight samples, the hardness definition provided in state water quality standards will be used to calculate the acute and chronic thresholds.

Appendix B

METHODS FOR ASSESSING COMPLIANCE WITH WQS USED FOR 303(d) LISTING PURPOSES: NUMERIC CRITERIA THAT ARE INCLUDED IN STATE WQS (10 CSR 20-7.031)

DESIGNATED USES	DATA TYPE	DATA QUALITY CODE	COMPLIANCE WITH WQS[i]	Notes
Protection of Aquatic Life	Lake nutrients (total phosphorus, total nitrogen, chlorophyll-a)	1-4	Full: Nutrient levels do not exceed WQS following procedures stated in Appendix D and F. Non-Attainment: Requirements for full attainment not met.	
Human Health - Fish Consumption	Chemical (water)	1-4	Full: Water quality does not exceed WQS following procedures stated in Appendix D. Non-Attainment: Requirements for full attainment not met.	
Drinking Water Supply (Raw)	Chemical (toxics)	1-4	Full: WQS not exceeded following procedures stated in Appendix D. Non-Attainment: Requirements for full attainment not met.	**Designated Use Note:** The Drinking Water Supply designated use defined in 10 CSR 20-7.031 applies to water bodies designated for such use.
Drinking Water Supply (Raw)	Chemical (sulfate, chloride, fluoride)	1-4	Full: WQS not exceeded following procedures stated in Appendix D. Non-Attainment: Requirements for full attainment not met.	
Drinking Water Supply (Finished)	Chemical (toxics)	1-4	Full: No Maximum Contaminant Level (MCL) violations based on Safe Drinking Water Act data evaluation procedures, in the most recent three years of available data. Non-Attainment: Requirements for full attainment not met.	**Compliance with WQS Note:** Finished water data will only be used for analytes listed in Table A1 of 10 CSR 20-7.031. Finished water data will not be used for analytes where water quality problems may be caused by the drinking water treatment process such as Trihalomethane (THM) formation, or problems that may be caused by distribution (bacteria, lead, copper).
Whole Body & Secondary Contact Recreation	*E. coli* count	2-4	Where there are at least five samples per year taken during the recreational season: Full: WQS not exceeded as a geometric mean, in any of the last three years for which data is available, for samples collected during seasons for which bacteria criteria apply.	**Compliance with WQS Note:** A geometric mean of 126, 206, and 1134 cfu/100 ml for *E. coli* will be used as a criterion for Category A, Category B, and Secondary Contact Recreational Waters.

Appendix B

METHODS FOR ASSESSING COMPLIANCE WITH WQS USED FOR 303(d) LISTING PURPOSES: NUMERIC CRITERIA THAT ARE INCLUDED IN STATE WQS (10 CSR 20-7.031)

DESIGNATED USES	DATA TYPE	DATA QUALITY CODE	COMPLIANCE WITH WQS[i]	Notes
			Non-Attainment: Requirements for full attainment not met.	
Whole Body Contact Recreation - Losing Streams	*E. coli* count	1-4	Full: No more than 10 percent of all samples exceed criterion. Non-Attainment: Requirements for full attainment not met. The criterion for *E. coli* is 126 counts/100 ml. 10 CSR 20-7.031 (5)(C)	
Irrigation, Livestock and Wildlife Water	Chemical (metals, fluoride)	1-4	Full: WQS not exceeded following procedures stated in Appendix D. Non-Attainment: Requirements for full attainment not met.	

[i] See section on Statistical Considerations, Appendix C and D.

Appendix C

METHODS FOR ASSESSING COMPLIANCE WITH WQS USED FOR 303(d) LISTING PURPOSES: NARRATIVE CRITERIA BASED ON NUMERIC THRESHOLDS NOT CONTAINED IN STATE WQS (10 CSR 20-7.031)

BENEFICIAL USES	DATA TYPE	DATA QUALITY CODE	COMPLIANCE WITH WATER QUALITY STANDARDS (WQS)[ii]	Notes
Overall use protection (all beneficial uses)	Narrative criteria for which quantifiable measurements can be made	1-4	Full: Stream condition typical of reference or appropriate regional control streams. Non-Attainment: Weight of evidence, based on the narrative criteria in 10 CSR 20-7.031(4), demonstrates the observed condition exceeds a numeric threshold necessary for the attainment of a beneficial use. *Color Example:* Color as measured by the Platinum-Cobalt visual method (SM 2120 B) in a water body is significantly higher (statistically) than a control water. *Objectionable Bottom Deposit Example:* The bottom covered by anthropogenic substances (e.g., sewage sludge, trash) exceeds the amount in reference or control streams by more than 20 percent. **Note:** Waters in mixing zones and unclassified waters that support aquatic life on an intermittent basis shall be subject to acute toxicity criteria for protection of aquatic life. Waters in the initial Zone of Dilution shall not be subject to acute toxicity criteria.	
Protection of Aquatic Life	Toxic Chemicals	1-4	Full: No more than one acute toxic event in three years (excluding natural die-offs of aquatic life). No more than one exceedance of acute or chronic criterion in three years for all toxins. Non-Attainment: Requirements for full attainment not met.	**Compliance with WQS Note**: Test result must be representative of water quality for the entire averaging period for which acute or chronic criteria apply. For ammonia, the chronic and acute exposure periods are 30 days and one hour, respectively. For all other toxics, the chronic and acute exposure periods are 96 hours and 24 hours, respectively. The Department will review all appropriate data, including hydrography, to ensure only representative data are used. Except on large rivers where storm water flows may persist at relatively unvarying levels for several days, grab samples

Appendix C

METHODS FOR ASSESSING COMPLIANCE WITH WQS USED FOR 303(d) LISTING PURPOSES: NARRATIVE CRITERIA BASED ON NUMERIC THRESHOLDS NOT CONTAINED IN STATE WQS (10 CSR 20-7.031)

BENEFICIAL USES	DATA TYPE	DATA QUALITY CODE	COMPLIANCE WITH WATER QUALITY STANDARDS (WQS)[ii]	Notes
Protection of Aquatic Life (cont.)	Toxic Chemicals (cont.)	See above.	See above.	collected during storm water flows will not be used for assessing chronic toxicity criteria, without additional corroborating evidence. **Compliance with WQS Note:** For toxic chemicals occurring in benthic sediment rather than water, the numeric thresholds used to determine the need for further evaluation will be the Probable Effect Concentrations (PECs) proposed by MacDonald *et al.* (2000).[1] These PEC thresholds are as follows: (in mg/kg) 33 As; 4.98 Cd; 111 Cr; 149 Cu; 48.6 Ni; 128 Pb; 459 Zn; and (in µg/kg) 22,800 total PAHs; 676 total PCBs; 17.6 chlordane ; 31.3 Sum DDE ; 4.99 lindane (gamma-BHC) . Where multiple sediment contaminants exist, the PECQ shall not exceed 0.75. See Section II. D for more on the PECQ.
Protection of Aquatic Life	Biological: Aquatic Macro-invertebrates sampled following Department Protocol	3-4	Full: For seven or fewer samples, 75 percent of stream condition index scores must be ≥16. Samples achieving these scores are considered very similar to regional reference streams. For greater than seven samples or for other sampling and evaluation protocols, results must be statistically similar to representative reference or control stream. Non-Attainment: For seven or fewer samples, 75 percent of stream condition index scores must be ≤14. Samples achieving these scores are considered substantially different from regional reference streams. For more than seven samples or for other sampling and evaluation protocols, results must be statistically dissimilar to control or representative reference streams.	**Data Type Note:** DNR macroinvertebrate protocol will not be used for assessment in the Mississippi Alluvial Basin (Bootheel area) due to lack of reference streams for comparison. **Data Type Note:** See Section II.D for additional criteria used to assess biological data. **Compliance with WQS Note:** See Appendix D. For test streams significantly smaller than biocriteria reference (bioreference) streams where both bioreference streams and small candidate reference streams are used to assess the test stream's biological integrity, the data assessment should display and consider both bioreference streams and candidate reference streams.

Appendix C

METHODS FOR ASSESSING COMPLIANCE WITH WQS USED FOR 303(d) LISTING PURPOSES: NARRATIVE CRITERIA BASED ON NUMERIC THRESHOLDS NOT CONTAINED IN STATE WQS (10 CSR 20-7.031)

BENEFICIAL USES	DATA TYPE	DATA QUALITY CODE	COMPLIANCE WITH WATER QUALITY STANDARDS (WQS)[ii]	Notes
Protection of Aquatic Life	Biological: MDC RAM Fish Community Protocol (Ozark Plateau only)	3-4	Full: For seven or fewer samples, 75 percent of fIBI scores must be ≥36. Samples achieving these scores are considered very similar to regional reference streams. For more than seven samples or for other sampling and evaluation protocols, results must be statistically similar to representative reference or control streams. Suspected of Impairment: Data inconclusive (Category 2B or 3B). For 1st and 2nd order streams, fIBI scores <29 Non-Attainment: 1st and 2nd order streams will not be assessed for non-attainment. When assessing 3rd to 5th order streams with seven or fewer samples, 75 percent of fIBI scores must be <36. Samples achieving these scores are considered substantially different from regional reference streams. For more than seven samples or for other sampling and evaluation protocols, results must be statistically dissimilar to control or representative reference streams.	**Data Type Note:** See Section II. D for additional criteria used to assess biological data. **Compliance with WQS Note:** MDC fIBI scores are from Doisy et al. (2008).[2] If habitat limitations (as measured by either the QCPH1 index or other appropriate methods) are judged to contribute to low fIBI scores and this is the only type of data available, the water body will be included in Category 4C, 2B, or 3B. If other types of data exist, the weight of evidence approach will be used as described in this LMD. **Compliance with WQS Note:** For determining influence of poor habitat on impaired samples, MDC RAM staff will be consulted. If, through this consultation, habitat is determined to be a significant probable cause for impairment, the water body will not be rated as impaired, but rather as suspected of impairment (Categories 2B or 3B). **Compliance with WQS Note:** See Appendix D. For test streams significantly smaller than bioreference streams where both bioreference streams and small candidate reference streams are used to assess the test stream's biological integrity, the data assessment should display and consider both biocriteria reference streams and candidate reference streams.
	Other Biological Data	3-4	Full: Results must be statistically similar to representative reference or control streams. Non-Attainment: Results must be statistically dissimilar to control or representative reference streams.	**Data Type Note:** See Section II. D for additional criteria used to assess biological data
	Toxicity testing using aquatic organisms (streams or lakes)	2	Full: No more than one test result of statistically significant deviation from controls in acute or chronic test in a three-year period. Non-Attainment: Requirements for full attainment not met.	

Appendix C

METHODS FOR ASSESSING COMPLIANCE WITH WQS USED FOR 303(d) LISTING PURPOSES: NARRATIVE CRITERIA BASED ON NUMERIC THRESHOLDS NOT CONTAINED IN STATE WQS (10 CSR 20-7.031)

BENEFICIAL USES	DATA TYPE	DATA QUALITY CODE	COMPLIANCE WITH WATER QUALITY STANDARDS (WQS)[ii]	Notes
Human Health - Fish Consumption	Chemicals (tissue)	1-2	Full: Contaminant levels in fish tissue (fillets, tissue plugs, eggs) do not exceed guidelines. Non-Attainment: Requirements for full attainment not met.	**Compliance with WQS Note**: Fish tissue thresholds are chlordane[3] 0.1 mg/kg; mercury[4] 0.3 mg/kg; PCBs[5] 0.75 mg/kg; and lead[6] 0.3 mg/kg. Assessment of mercury will be based on samples solely from the following higher trophic level fish species: walleye, sauger, trout, black bass, white bass, striped bass, northern pike, flathead catfish and blue catfish.

[ii] See section on Statistical Considerations and Appendix D.

[1] MacDonald, D.D, Ingersoll, C. G., Berger, T. A. 2000. Development and evaluation of consensus-based sediment quality guidelines for freshwater ecosystems. Arch. Environ. Contamination Toxicology. 39, 20-31.

[2] Doisy, K.E., C.F. Rabeni, M.D. Combes, and R.J. Sarver. 2008. Biological criteria for stream fish communities of Missouri. Final Report to the United States Environmental Protection Agency. Missouri Cooperative Fish and Wildlife Research Unit, Columbia, MO. 91.

[3] Crellin, J.R. 1989. *"New Trigger Levels for Chlordane in Fish-Revised Memo."* MO Dept of Health inter-office memorandum. June 16, 1989

[4] US EPA. 2001. Water quality criterion for protection of human health: methylmercury. EPA-823-R-01-001. https://www.epa.gov/sites/default/files/2020-01/documents/methylmercury-criterion-2001.pdf

[5] DHSS. 2006. *"Development of PCB Risk-based Fish Consumption Limit Tables."* MO Dept of Health and Senior Services Memorandum. August 30, 2006.

[6] World Health Organization. 1972. *"Evaluation of Certain Food Additives and the Contaminants Mercury, Lead and Cadmium."* WHO Technical Report Series No. 505, Sixteenth Report on the Joint FAO/WHO Expert Committee on Food Additives. Geneva. 33.

Appendix D

DESCRIPTION OF ANALYTICAL TOOLS USED FOR DETERMINING THE STATUS OF MISSOURI WATERS (11" X 14" FOLD OUT)

			Determining when waters are impaired			Determining when waters are no longer impaired			
Designated Use	**Analytes**	**Analytical Tool**	**Decision Rule/ Hypothesis**	**Criterion Used with the Decision Rule[iii]**	**Significance Level (α)**	**Decision Rule/ Hypothesis**	**Criterion Used with the Decision Rule**	**Significance Level (α)**	**Notes**
Narrative Criteria	Color	Hypothesis Test, two sample, one tailed t-Test	Null Hypothesis: No difference in color between test stream and control stream.	Reject Null Hypothesis if calculated "t" value exceeds tabular "t" value for test alpha	0.1	Same Hypothesis	Same Criterion	Same Significance Level	
	Objectionable bottom deposits	Hypothesis Test, two sample, one tailed t-Test	Null Hypothesis: Solids of anthropogenic origin cover is no greater than that of the control plus 20 percent.	Reject Null Hypothesis if 60 percent Lower Confidence Limit (LCL) of mean percent fine sediment deposition (pfsd) in stream is greater than the sum of the pfsd in the control plus 20 percent more of the stream bottom.	0.4	Same Hypothesis	Same Criterion	Same Significance Level	**Criterion Note:** Every effort should be made to estimate sediment deposition when average stream velocity is less than 0.5 feet per second. Fine sediment is defined as sediment particles less than 2mm in size. If data is non-normal a nonparametric test will be used as a comparison of medians. The 20 percent stream bottom coverage, greater than control still applies. Example: Average control stream percent fine sediment deposition is equal to 5%. Test stream average percent fine sediment deposition would be compared to 25% (5% plus 20%).

Appendix D

DESCRIPTION OF ANALYTICAL TOOLS USED FOR DETERMINING THE STATUS OF MISSOURI WATERS (11" X 14" FOLD OUT)

			Determining when waters are impaired			Determining when waters are no longer impaired			
Designated Use	**Analytes**	**Analytical Tool**	**Decision Rule/ Hypothesis**	**Criterion Used with the Decision Rule[iii]**	**Significance Level (α)**	**Decision Rule/ Hypothesis**	**Criterion Used with the Decision Rule**	**Significance Level (α)**	**Notes**
	Filamentous algae	Hypothesis Test, two sample, one tailed t-Test	Null Hypothesis: Filamentous algae cover is no greater than that of the control plus 20 percent.	Reject Null Hypothesis if 60 percent Lower Confidence Limit (LCL) of mean percent filamentous algae coverage in stream is greater than the sum of the percent filamentous algae coverage in the control plus 20 percent more of the stream bottom.	0.4	Same Hypothesis	Same Criterion	Same Significance Level	**Criterion Note:** If data is non-normal a nonparametric test will be used as a comparison of medians. The 20 percent stream bottom coverage, greater than control still applies. Example: Average control stream percent bottom coverage of filamentous algae is equal to 5%. Test stream percent bottom coverage of filamentous algae would be compared to 25% (5% plus 20%). Control streams must be sampled in conjunction with the test streams.
Aquatic Life	Biological monitoring (Narrative)	DNR Invert protocol: sample size of 7 or less, 75 percent of samples must score 14 or lower or RAM fIBI protocol: sample size of 7 or less, 75 percent of samples must score <36	Using DNR Invert Protocol, Null Hypothesis: Frequency of full sustaining scores for test stream is the same as biocriteria reference streams	Reject Null Hypothesis if frequency of fully sustaining scores on test stream is significantly less than for biocriteria reference streams	N/A	Same Hypothesis	Same Criterion	Same Significance Level	

Appendix D

DESCRIPTION OF ANALYTICAL TOOLS USED FOR DETERMINING THE STATUS OF MISSOURI WATERS (11" X 14" FOLD OUT)

			Determining when waters are impaired			Determining when waters are no longer impaired			
Designated Use	**Analytes**	**Analytical Tool**	**Decision Rule/ Hypothesis**	**Criterion Used with the Decision Rule[iii]**	**Significance Level (α)**	**Decision Rule/ Hypothesis**	**Criterion Used with the Decision Rule**	**Significance Level (α)**	**Notes**
Aquatic Life (cont.)	Biological monitoring (Narrative)	DNR Invert protocol OR RAM fIBI protocol with sample sizes of 8 or more: Binomial Probability	A direct comparison of frequencies between test and biocriteria reference streams will be made.	Rate as impaired if the frequency of biocriteria reference streams with fully supporting biological scores is greater than five percent more than test stream.	0.1	Same Hypothesis	Same Criterion	Same Significance Level	**Criterion Note:** For inverts, the reference number will change depending on which EDU the stream is in (X percent - 5 percentage points), for RAM samples the reference number will always be 70 (75 percent - 5 percentage points).
		For other biological data an appropriate parametric or nonparametric test will be used	Null Hypothesis: Community metric(s) in test stream is the same as in a reference stream or control stream.	Reject Null Hypothesis if metric scores for test stream are significantly less than reference or control streams.	0.1	Same Hypothesis	Same Criterion	Same Significance Level	
			Other biological monitoring to be determined by type of data.	Dependent upon available information	Dependent upon available information	Same Hypothesis	Same Criterion	Same Significance Level	

Appendix D

DESCRIPTION OF ANALYTICAL TOOLS USED FOR DETERMINING THE STATUS OF MISSOURI WATERS (11" X 14" FOLD OUT)

			Determining when waters are impaired			Determining when waters are no longer impaired			
Designated Use	**Analytes**	**Analytical Tool**	**Decision Rule/ Hypothesis**	**Criterion Used with the Decision Rule[iii]**	**Significance Level (α)**	**Decision Rule/ Hypothesis**	**Criterion Used with the Decision Rule**	**Significance Level (α)**	**Notes**
Aquatic Life (cont.)	Toxic chemicals, water (Numeric)	Not applicable (N/A)	No more than one toxic event, toxicity test failure or exceedance of acute or chronic criterion in 3 years.	N/A	N/A	N/A	N/A	N/A	
	Toxic chemicals, sediment (Narrative)	Comparison of geometric mean to PEC value, or calculation of a PECQ value.	Parameter geomean exceeds PEC, or site PECQ is exceeded.	For metals, use 150 percent PEC threshold. The PECQ threshold value is 0.75.	N/A	Parameter geomean is equal to or less than PEC, or site PECQ equaled or not exceeded.	For metals, use 150 percent of PEC threshold. The PECQ threshold value is 0.75.	N/A	**Compliance with Water Quality Standards Note:** In the case of toxic chemicals occurring in benthic sediment rather than in water, the numeric thresholds used to determine the need for further evaluation will be the Probable Effect Concentrations (PECs) proposed by MacDonald *et al.* (2000).[1] These PECs are as follows (in mg/kg): 33 As; 4.98 Cd; 111 Cr; 149 Cu; 48.6 Ni; 128 Pb; 459 Zn; and (in µg/kg) ; 22,800 total PAHs; 676 total PCBs; 17.6 chlordane; 31.3 Sum DDE; 4.99 lindane (gamma-BHC). Where multiple sediment contaminants exist, the PECQ shall not exceed 0.75. See Section II. D for more information on PECQs.
	Temperature, pH, total dissolved gases, oil and grease, dissolved oxygen (Numeric)	Binomial probability	Null Hypothesis: No more than 10 percent of samples exceed the water quality criterion.	Reject Null Hypothesis if the Type I error rate is less than 0.1.	N/A	Same Hypothesis	Same Criterion	N/A	Data collected will be treated as is and the binomial probability calculation will be used for assessment.

Appendix D

DESCRIPTION OF ANALYTICAL TOOLS USED FOR DETERMINING THE STATUS OF MISSOURI WATERS (11" X 14" FOLD OUT)

			Determining when waters are impaired			Determining when waters are no longer impaired			
Designated Use	**Analytes**	**Analytical Tool**	**Decision Rule/ Hypothesis**	**Criterion Used with the Decision Rule[iii]**	**Significance Level (α)**	**Decision Rule/ Hypothesis**	**Criterion Used with the Decision Rule**	**Significance Level (α)**	**Notes**
Human Health – Fish Consumption	Toxic chemicals, water (Numeric)	Hypothesis test: 1-sided confidence limit	Null Hypothesis: Contaminant levels in water do not exceed criterion.	Reject Null Hypothesis if the 60 percent LCL is greater than the criterion value.	0.4	Same Hypothesis	Reject Null Hypothesis if the 60 percent UCL is greater than the criterion value.	Same Significance Level	
Human Health – Fish Consumption	Toxic chemicals, tissue (Narrative)	Four or more samples: Hypothesis test 1-sided confidence limit	Null Hypothesis: Contaminant levels in fillets or eggs do not exceed criterion.	Reject Null Hypothesis if the 60 percent LCL is greater than the criterion value.	0.4	Same Hypothesis	Reject null hypothesis if the 60 percent UCL is greater than the criterion value.	Same Significance Level	
Drinking Water Supply (Raw)	Toxic chemicals (Numeric)	Hypothesis test: 1-sided confidence limit	Null Hypothesis: Contaminant levels do not exceed criterion.	Reject Null Hypothesis if the 60 percent LCL is greater than the criterion value.	0.4	Same Hypothesis	Reject null hypothesis if the 60 percent UCL is greater than the criterion value.	Same Significance Level	
	Non-toxic chemicals (Numeric)	Hypothesis test: 1-sided confidence limit	Null Hypothesis: Contaminant levels do not exceed criterion.	Reject Null Hypothesis: if the 60 percent LCL is greater than the criterion value.	0.4	Same Hypothesis	Reject null hypothesis if the 60 percent UCL is greater than the criterion value.	Same Significance Level	

Appendix D

DESCRIPTION OF ANALYTICAL TOOLS USED FOR DETERMINING THE STATUS OF MISSOURI WATERS (11" X 14" FOLD OUT)

			Determining when waters are impaired			Determining when waters are no longer impaired			
Designated Use	**Analytes**	**Analytical Tool**	**Decision Rule/ Hypothesis**	**Criterion Used with the Decision Rule[iii]**	**Significance Level (α)**	**Decision Rule/ Hypothesis**	**Criterion Used with the Decision Rule**	**Significance Level (α)**	**Notes**
Drinking Water Supply (Finished)	Toxic chemicals	Stipulated by Safe Drinking Water Act	No MCL violations in most recent three years.	N/A	N/A	N/A	N/A	N/A	**Compliance with WQS Note:** Finished water data will only be used for analytes listed in Table A1 of 10 CSR 20-7.031. Finished water data will not be used for analytes where water quality problems may be caused by the drinking water treatment process such as Trihalomethane (THM) formation, or problems that may be caused by distribution (bacteria, lead, copper).
Whole Body & Secondary Contact Recreation	Bacteria (Numeric)	Geometric mean	Null Hypothesis: Contaminant levels do not exceed criterion.	Reject Null Hypothesis: if the geometric mean is greater than the criterion value.	N/A	Same Hypothesis	Same Criterion	N/A	
Losing Streams	*E.coli*	Binomial probability	Null Hypothesis: No more than 10 percent of samples exceed the water quality criterion.	Reject Null Hypothesis if the Type I error rate is <0.1.	0.1	Same Hypothesis	Same Criterion	Same Significance Level	
Irrigation & Livestock Water	Toxic chemicals (Numeric)	Hypothesis test 1-Sided confidence limit	Null Hypothesis: Contaminant levels do not exceed criterion.	Reject Null Hypothesis if the 60 percent LCL is greater than the criterion value.	0.4	Same Hypothesis	Reject null hypothesis if the 60 percent UCL is greater than the criterion value.	Same Significance Level	

Appendix D

DESCRIPTION OF ANALYTICAL TOOLS USED FOR DETERMINING THE STATUS OF MISSOURI WATERS (11" X 14" FOLD OUT)

			Determining when waters are impaired			Determining when waters are no longer impaired			
Designated Use	**Analytes**	**Analytical Tool**	**Decision Rule/ Hypothesis**	**Criterion Used with the Decision Rule[iii]**	**Significance Level (α)**	**Decision Rule/ Hypothesis**	**Criterion Used with the Decision Rule**	**Significance Level (α)**	**Notes**
Protection of Aquatic Life	Lake nutrients (Numeric – Site Specific)	Hypothesis test	Null hypothesis: Criteria are not exceeded.	Reject Null Hypothesis if 60 percent LCL value is greater than criterion value.	0.4	Same Hypothesis	Same Criterion	Same Significance Level	**Hypothesis Test Note:** State nutrient criteria require at least four samples per year taken near the outflow point of the lake (or reservoir) between May 1 and September 30 for at least four different, not necessarily consecutive, years.
	Lake nutrients (Numeric – Ecoregional)	Geometric mean	Null Hypothesis: Contaminant levels do not exceed criterion.	Reject Null Hypothesis: if the geometric mean is greater than the criterion value.	N/A	Same Hypothesis	Same Criterion	N/A	See Appendix F for more information.

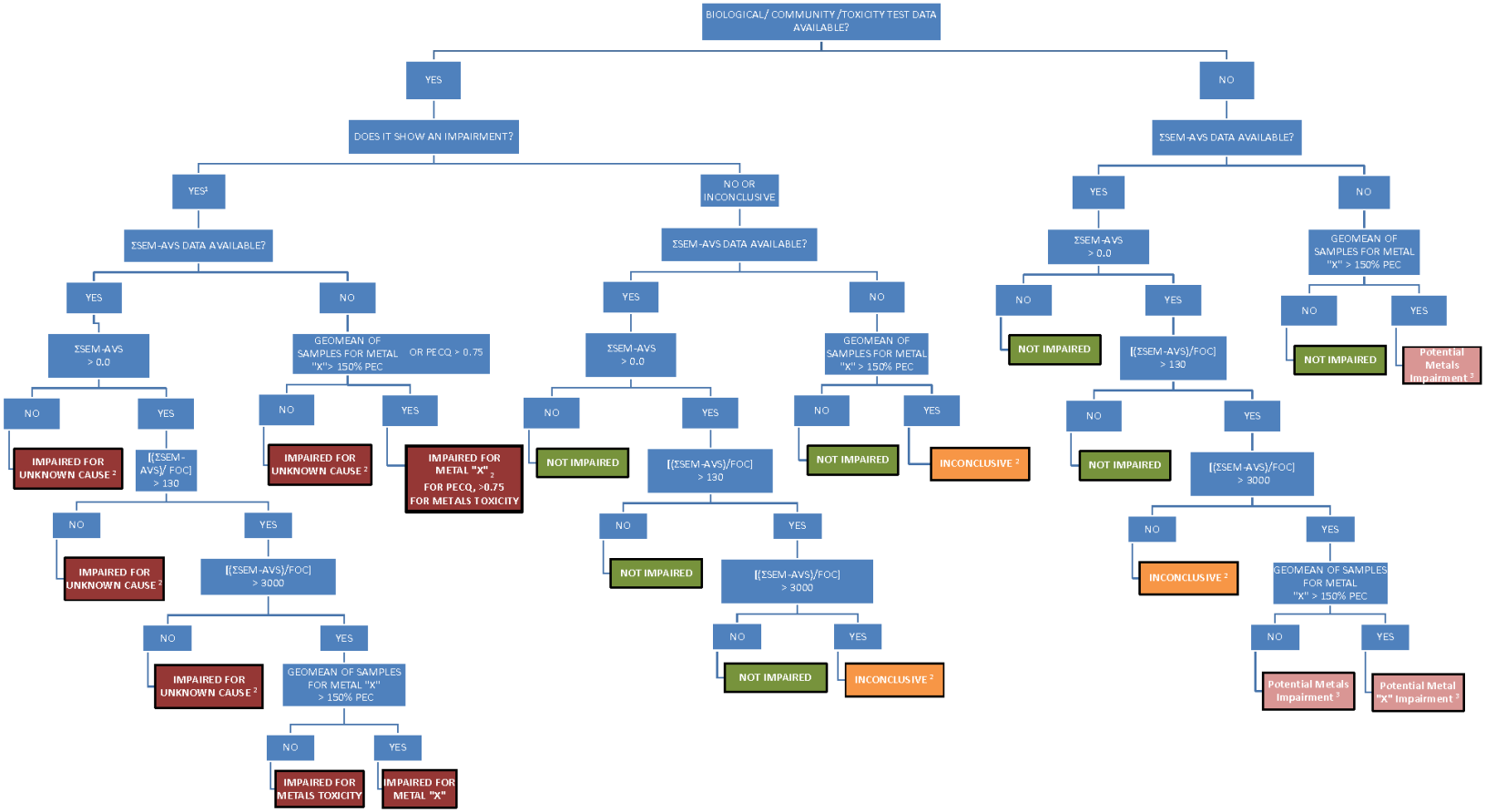
[iii] Where hypothesis testing is used for data sets with five samples or fewer (for media other than fish tissue), a 75 percent confidence interval around the appropriate central tendencies will be used to determine use attainment status. Use attainment will be determined as follows: (1) If the criterion value is above this interval (all values within the interval are in agreement with the criterion), rate as unimpaired; (2) If the criterion value falls within this interval, rate as unimpaired and place in Category 2B or 3B; (3) If the criterion value is below this interval (all values within the interval are not in agreement with the criterion), rate as impaired. For fish tissue, this procedure will be used with the following amendments: (1) it will apply only to sample sizes of less than four and, (2) a 50 percent confidence interval will be used in place of the 75 percent confidence interval.

[1] MacDonald, D.D, Ingersoll, C. G., Berger, T. A. 2000. Development and Evaluation of Consensus-Based Sediment Quality Guidelines for Freshwater Ecosystems. Arch. Environ. Contamination Toxicology. 39, 20-31.

Appendix E

PICTORIAL REPRESENTATIONS OF THE WEIGHT OF EVIDENCE PROCEDURE FOR JUDGING TOXICITY OF SEDIMENT DUE TO METALS AND PAHS

Biological Weight of Evidence Decision Chart - Sediment Toxicity (Metals)



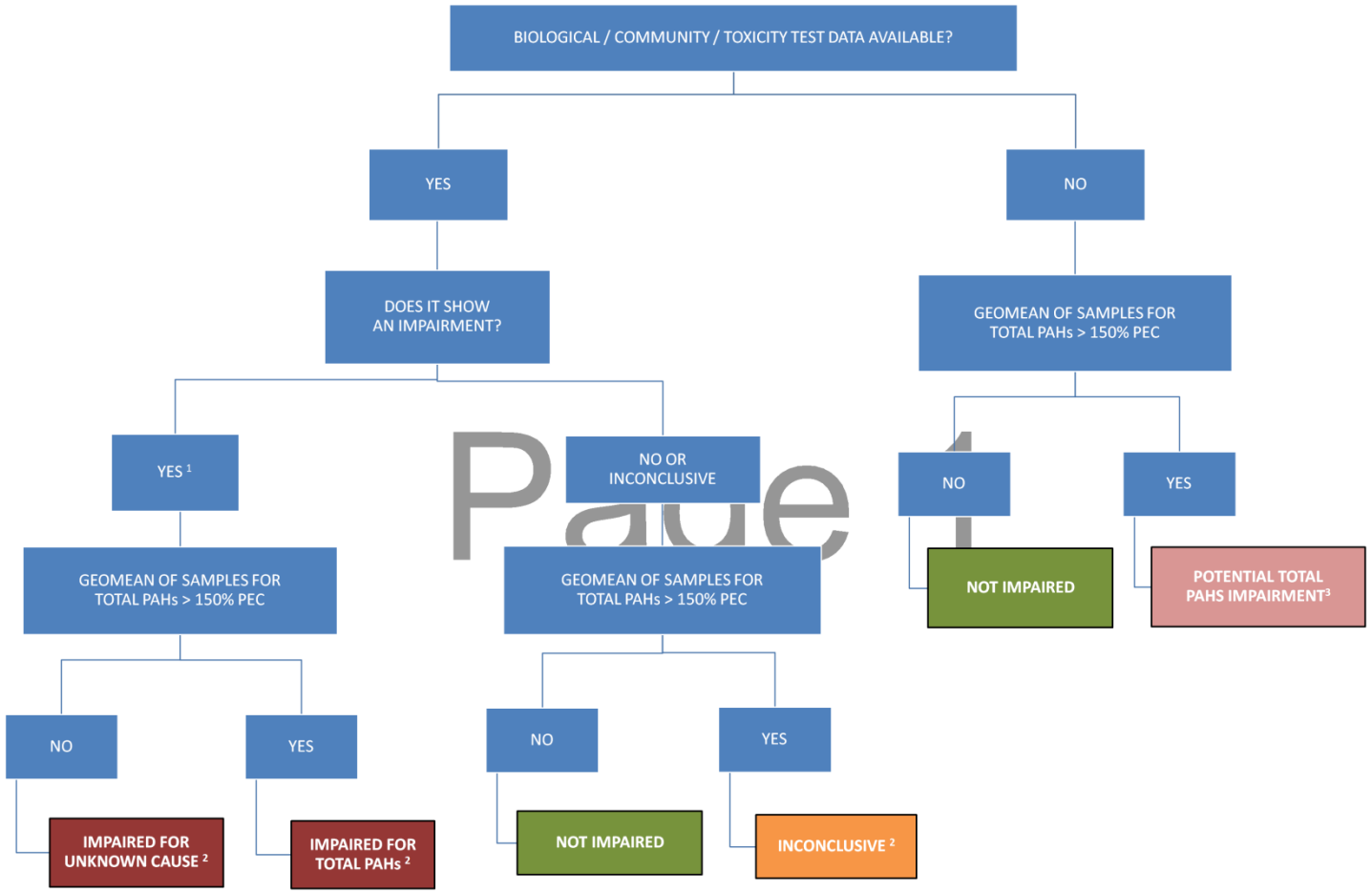
Notes:

1 - If there are Numeric WQS violations (unrelated to sediment) then follow LMD Procedure in LMD Appendix B. **Do Not Continue.**

2 - Note waterbody for further investigation related to metals or habitat issues.

3 - Note waterbody for Biological Sampling.

Biological Weight of Evidence Decision Chart - Sediment Toxicity (PAHs)



Notes:

1 - If there are Numeric WQS violations (unrelated to sediment) then follow LMD Procedure in LMD Appendix B. Do Not Continue.

2 - Note waterbody for further investigation.

3 - Note waterbody for Biological Sampling.

Appendix F

Missouri's Nutrient Criteria

Missouri Lakes and Reservoirs

For the purposes of Missouri's nutrient criteria and this document, all lakes and reservoirs are referred to as "lakes" [10 CSR 20-7.031(5)(N)1.A.]. Missouri's lakes are more appropriately classified as impoundments and have very different physical, chemical, and biological characteristics when compared to naturally-formed glacial or mountainous lakes found in other states. Many of Missouri's major lakes were constructed primarily for flood control, hydroelectric power, and water supply. The riverine habitats and species that existed before impoundment over time transitioned into the current state of aquatic life dominated by self-sustaining populations of sport and non-sport fishes. The numeric nutrient criteria and implementation methods proposed by the Department are structured to ensure the deleterious impacts of nutrient enrichment to Missouri's lakes are mitigated without adverse impacts to the health and vitality of the self-sustaining populations of aquatic life that live there.

Missouri's nutrient criteria apply to all lakes that are waters of the state and have an area of at least 10 acres during normal pool condition, except the natural lakes (oxbows) in the Big River Floodplain ecoregion [10 CSR 20-7.031(5)(N)2.]. The criteria apply to, and assessments will be conducted for, the entire water body as found in Missouri's WQS regulation. As noted in the *Rationale for Missouri Lake Nutrient Criteria* (DNR, 2017), the Department has structured Missouri's nutrient criteria as a decision framework that applies at an ecoregional basis. This decision framework integrates causal and response parameters into one WQS that accounts for uncertainty in linkages between causal and response parameters. The decision framework includes response impairment thresholds, nutrient screening thresholds, and response assessment endpoints. This framework appropriately integrates causal and response parameters and is based on the bioconfirmation guiding principles that EPA (2013) has suggested as an approach for developing nutrient criteria.

Numeric Criteria for Lakes [10 CSR 20-7.031(5)(N)]

Missouri's WQS contain response impairment threshold values for chlorophyll-a (Chl-a) and screening threshold values for total nitrogen (TN), total phosphorus (TP), and Chl-a, all of which vary by the dominant watershed ecoregion. Lakes are determined to be impaired if the geometric mean of samples taken between May and September in a calendar year exceeds the Chl-a response impairment threshold value more than once in three years' time. A duration of three or more years is necessary to account for natural variations in nutrient levels due to climatic variability (Jones and Knowlton, 2005). If a lake exceeds a screening threshold value, it will be designated as impaired if any of five response assessment endpoints also are identified in the same calendar year.

Lake Ecoregion	Chl-a Response Impairment Thresholds (µg/L)	Nutrient Screening Thresholds (µg/L)		
		TP	TN	Chl-a
Plains	30	49	843	18
Ozark Boarder	22	40	733	13
Ozark Highland	15	16	401	6

The five response assessment endpoints are:

- Occurrence of eutrophication-related mortality or morbidity events for fish and other aquatic organisms
- Epilimnetic excursions from dissolved oxygen or pH criteria
- Cyanobacteria counts in excess of 100,000 cells/mL
- Observed shifts in aquatic diversity attributed to eutrophication
- Excessive levels of mineral turbidity that consistently limit algal productivity during the period of May 1 – September 30

All scientific references used for numeric nutrient criteria derivation are contained in the *Rationale for Missouri Lake Nutrient Criteria* (DNR, 2017) and supplemental materials maintained by the Department. The Department maintains a copy of these references and makes them available to the public for inspection and copying at no more than the actual cost of reproduction.

Narrative Criteria [10 CSR 20-7.031(4)]

Missouri's WQS contain general (narrative) water quality criteria that are used to protect waters from nutrient enrichment caused by excessive nitrogen and/or phosphorous loading. Missouri's general criteria protect waters from "unsightly or harmful bottom deposits" and "unsightly color or turbidity," which are potential consequences of excess nutrients in freshwater systems. Narrative criteria do not provide numeric thresholds or concentrations above which impacts to designated uses are likely to occur. However, because the bioconfirmation approach integrates causal and response variables to ensure attainment of the aquatic habitat protection use, the proposed numeric nutrient criteria and screening thresholds serve as an enforceable interpretation of Missouri's general criteria at 10 CSR 20-7.031(4). Additionally, implementation of the numeric nutrient criteria and screening thresholds also will ensure protection of downstream waters as required by 10 CSR 20-7.031(4)(E) and 40 CFR 131.10(b).

Site-Specific Numeric Criteria [10 CSR 20-7.031(5)(N)]

Missouri's WQS also contain numeric nutrient criteria for specific lakes. Each of the lakes listed in Table N of the WQS have site-specific criteria for TN, TP, and Chl-a, based on the annual geometric mean of a minimum of three years of data and characteristics of the lake. Additional site-specific criteria may be developed to account for the unique characteristics of a water body.

Data Requirements for Assessment

In order to assess a lake against the numeric nutrient criteria in 10 CSR 20-7.031(5)(N), the following data requirements must be met:

1. At least four samples collected between May 1 and September 30 under representative conditions, collected near the dam or deepest part of the reservoir, and collected within a half meter of the surface;
2. Each sample must have been analyzed for at least Chl-a, TN, TP, and Secchi depth;
3. At least three years of samples (years do not have to be consecutive). Data older than seven years will be used consistent with the LMD;
4. Data collected under a QAPP.

If these requirements are not met, the lake will be placed into Category 3 of Missouri's Integrated Water Quality Report (i.e., Missouri's 305(b) Report) until further information can be collected. In the case of lakes that have some data, but not enough to make an assessment, these lakes will be prioritized for additional sampling. Lakes with limited data where water quality trends or field observations point to possible impairment will receive the highest priority.

Criteria for Assessment

Each lake will be evaluated against the appropriate ecoregional or site-specific criteria located in Tables L, M, and N of 10 CSR 20-7.031 (reproduced below).

Table L: Lake Ecoregion Chl-a Response Impairment Threshold Values (µg/L)

Lake Ecoregion	Chl-a Response Impairment Thresholds
Plains	30
Ozark Border	22
Ozark Highland	15

Table M: Lake Ecoregion Nutrient Screening Threshold Values (µg/L)

Lake Ecoregion	Nutrient Screening Thresholds		
	TP	TN	Chl-a
Plains	49	843	18
Ozark Border	40	733	13
Ozark Highland	16	401	6

Table N: Site-Specific Nutrient Criteria

Lake Ecoregion	Lake	County	Site-Specific Criteria (µg/L)		
			TP	TN	Chl-a
Plains	Bowling Green Lake	Pike	21	502	6.5
	Bowling Green Lake (old)	Pike	31	506	5
	Forest Lake	Adair	21	412	4.3
	Fox Valley Lake	Clark	17	581	6.3
	Hazel Creek Lake	Adair	27	616	6.9
	Lincoln Lake – Cuivre River State Park	Lincoln	16	413	4.3
	Marie, Lake	Mercer	14	444	3.6
	Nehai Tonkaia Lake	Chariton	15	418	2.7
	Viking, Lake	Daviess	25	509	7.8
	Waukomis Lake	Platte	25	553	11
	Weatherby Lake	Platte	16	363	5.1

Ozark Border	Goose Creek Lake	St Francois	12	383	3.2
	Wauwanoka, Lake	Jefferson	12	384	6.1
Ozark Highland	Clearwater Lake	Wayne-Reynolds	13	220	2.6
	Council Bluff Lake	Iron	7	229	2.1
	Crane Lake	Iron	9	240	2.6
	Fourche Lake	Ripley	9	236	2.1
	Loggers Lake	Shannon	9	200	2.6
	Lower Taum Sauk Lake	Reynolds	9	203	2.6
	Noblett Lake	Douglas	9	211	2
	St. Joe State Park Lakes	St Francois	9	253	2
	Sunnen Lake	Washington	9	274	2.6
	Table Rock Lake	Stone	9	253	2.6
	Terre du Lac Lakes	St Francois	9	284	1.7
	Timberline Lakes	St Francois	8	276	1.5

Assessment Methodology

1. Site-Specific Lake Nutrient Criteria
   Lakes with site-specific numeric nutrient criteria (see Table N of 10 CSR 20-7.031) will be assessed using the current listing methodology.

2. Ecoregional Lake Nutrient Criteria
   Lakes with ecoregional nutrient criteria (see Tables L and M of 10 CSR 20-7.031) will be assessed using the following:

   a. For lakes with ecoregional criteria, a yearly geometric mean for Chl-a, TN, and TP will be calculated for the period of record. The latest three years (do not have to be consecutive) of data will be used for assessment. These data are collected by the SLAP and the LMVP.

   b. If the geometric mean of Chl-a exceeds the response impairment threshold in more than one of the latest three years of available data, the lake will be placed into Category 5 and go on Missouri's 303(d) list for Chl-a. If only two years of data are available and the geometric mean of Chl-a exceeds the response impairment threshold in both years, the lake will be placed into Category 5 and go on Missouri's 303(d) list for Chl-a.

   c. If the geometric mean of Chl-a, TN, or TP exceeds the nutrient screening threshold, then additional response assessment endpoints will be evaluated (see Assessment Methodology Section #3 "Additional Lake Response Assessment Endpoints" below). If data for any of the response assessment endpoints indicates impairment in the same year that Chl-a, TN, or TP exceeds the nutrient screening threshold, the lake will be placed Category 5 and go on Missouri's 303(d) list for Chl-a. If sufficient data are not available to assess the response assessment endpoints or they do not show impairment, then the water will be placed into Category 3B or 2B, respectively (assuming other uses are attaining) and prioritized for additional monitoring and ongoing evaluation of response assessment endpoints (see Monitoring Efforts Section). The Department is committed to providing the data needed to complete the full assessment.

d. If the geometric mean of Chl-a, TN, or TP does not exceed the nutrient screening threshold, the water will be placed into the appropriate IR category based on the attainment of the other uses.

e. The period of record for the lake will be reviewed for the purpose of determining long-term trends in water quality. If a lake is determined to be trending towards potential impairment, the lake will be further scrutinized and prioritized for additional monitoring.

3. Additional Lake Response Assessment Endpoints

For lakes where the geometric mean of Chl-a, TN, or TP exceeds the ecoregional nutrient screening thresholds, the additional response assessment endpoints listed below will be evaluated. Each of these endpoints is linked to the protection of the aquatic habitat designated use and will be used to assess compliance with the numeric nutrient criteria when screening values are exceeded. When one of these endpoints indicate a eutrophication impact in the same year as a nutrient screening threshold exceedance, the lake will be placed into Category 5 and on the 303(d) list.

Response assessment endpoints observed in lakes without sufficient data for Chl-a, TP, or TN will be prioritized highest for additional sampling of Chl-a, TP, and TN.

a. 10 CSR 20-7.031(5)(N)6.A. – Occurrence of eutrophication-related mortality or morbidity events for fish and other aquatic organisms (i.e., fish kills)

- Following the Department's LMD, two or more fish kills within the last three years of available data will result in the water being placed into Category 5 as well as the 303(d) list.

- Fish kills as a result of nutrient enrichment (eutrophication) in a lake indicate that current water quality may not be protective of the aquatic habitat designated use. The Department maintains contact with the MDC on fish kills that occur throughout the state. MDC, as well as the Department's Environmental Emergency Response and Water Protection Program, receive notifications of observed fish kills. MDC investigates reported fish kills and provides a summary report of the species, size, and number of fish and other aquatic organisms killed. These reports are provided shortly after the investigation. Annual fish kill reports are compiled and provided to the Department, only fish kills that have been verified by agency staff will be used as evidence of impairment.

  The Department will continue to request these data and annual reports from MDC. This document includes fish kill data and causes as well as describes the methods used by MDC to assess fish kills.

- The Department will review reports for information pertaining to the cause of death as well as the potential sources. Fish populations can have seemingly random small die-offs related to disease, virus, or other natural causes. The Department will focus on die-offs related to DO, temperature, pH, algal blooms, and the toxins associated with algal blooms. More than one fish kill within 10 years or one large (>100 fish and covering more than ten percent of the lake area) fish kill documented to be caused by dissolved oxygen excursions, pH, algal blooms, or the toxins associated with algal blooms will constitute evidence of impairment.

b. 10 CSR 20-7.031(5)(N)6.B. – Epilimnetic excursions from dissolved oxygen or pH criteria

In lakes, DO is produced by atmospheric reaeration and the photosynthetic activity of aquatic plants and consumed through respiration. DO production by aquatic plants (primarily phytoplankton in Missouri reservoirs) is limited to the euphotic zone where sufficient light exists to support photosynthesis. In some lakes, reaeration and photosynthesis may be sufficient to support high DO levels throughout the water column during periods of complete mixing. Missouri lakes however, do not stay completely mixed and thermally stratify during the summer. The duration, depth, and areal extent of stratification in any lake is a function of site-specific lake variables and environmental factors. During the stratified period, the epilimnion (surface water layer) receives oxygen from the atmosphere and is dominated by primary production from phytoplankton and other aquatic plants. In contrast, the hypolimnion (deep, cool water zone) is largely separated from the epilimnion (surface layer) and is dominated by respiratory processes that use organic matter derived from autochthonous (in-lake) and allochthonous (watershed) sources. The strong temperature gradient between the epilimnion and hypolimnion generally restricts gas and nutrient circulation and limits the movement of phytoplankton between the layers. As a result, respiration in the hypolimnion creates hypoxic conditions during the stratification period.

Data collected by the MU demonstrates that hypoxic hypolimnetic conditions (DO less than 2 mg/l) consistently occur during the summer in Missouri lakes regardless of trophic condition. Further, anoxic hypolimnetic conditions (absence of dissolved oxygen) have even been measured in Missouri's high-quality oligotrophic lakes. It is apparent from the science and available data that low hypolimnetic DO conditions are the result of natural processes and should be expected in all lakes across the state. Thermal stratification and resulting hypoxic or anoxic hypolimnia limit the area where some more sensitive fish species thrive to the epilimnion. Assessment of DO in the epilimnion of lakes will ensure the protection of aquatic life and aquatic habitat designated use and the maintenance of a robust aquatic community. Therefore, it would be inappropriate to apply the 5.0 milligrams per liter DO criterion throughout the entire water column.

DO and pH criterion will apply only to the epilimnion during thermal stratification. DO and pH criteria will apply throughout the water column outside of thermal stratification.

Excess nutrient input into lakes causes an increase in primary productivity of a lake. This increase in productivity comes with an increasing demand for DO through both the living and the decaying portions of aquatic life. Increased productivity also causes algal populations to have exponential growth and decay rates that can cause swings in DO concentrations. Sudden drops in DO concentrations or low levels of DO concentrations can cause fish kills.

Similar to DO, water column pH levels are linked to photosynthesis and impacted by thermal stratification. During periods of high photosynthesis, carbon dioxide (CO_2) is removed from the water column and pH increases. Conversely, when respiration and decomposition is high, CO_2 levels increase and pH decreases. As described above, the natural temperature gradients during the summer growing season create conditions whereby the epilimnion is dominated by primary production and the hypolimnion is dominated by respiration. Therefore, the pH levels will typically be higher in the epilimnion and lower in the hypolimnion. Because the nutrient criteria are focused on the biological response variable Chl-a, which is highest in the epilimnion in the summer, it is appropriate to limit pH assessments to the epilimnion.

Excessive algal production can cause the pH of the epilimnion to rise above 9.0 in some cases. When pH falls outside of this range due to algal blooms and their eventual decomposition, aquatic life which requires a stable range of pH conditions to survive can suffer. As mentioned for dissolved oxygen, assessment of pH in the epilimnion of lakes against WQS will ensure the protection of aquatic life and the aquatic habitat designated use, and the maintenance of a robust aquatic community.

- At the time of sample collection, DO, water temperature, and pH will be measured near the surface as well as via sonde probe throughout the depth of the epilimnion (water surface to the thermocline). The sonde probe continuously collects data for a short period of time as it is lowered through the water column. This data is currently collected by the SLAP.

- Following the LMD procedure for DO: If more than 10 percent of the measurements are below the 5.0 mg/L minimum to protect aquatic life, the binomial probability will be used for to determine whether the criterion has been exceeded.

- Following the LMD procedure for pH: If more than 10 percent of the measurements are outside the 6.5 to 9.0 range to protect aquatic life, the binomial probability will be used to determine whether the criterion has been exceeded.

c. 10 CSR 20-7.031(5)(N)6.C. – Cyanobacteria counts in excess of one hundred thousand (100,000) cells per milliliter (cells/mL)

Cell counts of cyanobacteria (blue-green algae) greater than 100,000 can be indicative of a harmful algal bloom (HAB) and the increased probability of algal toxins in the lake. Certain species of blue-green algae can produce toxins harmful to both aquatic life and terrestrial life (including humans and pets). *Microcystis* can produce microcystin (liver toxin) and anatoxin-a (neurotoxin). *Dolichospermum*, in addition to producing microcystin and anatoxin-a, also can produce cylindrospermopsin (liver toxin) and saxitoxin (nerve toxin). These toxins can cause adverse effects on aquatic life, as well as humans recreating on surface waters. The Oregon Health Authority has developed recreational guidelines for issuing public health advisories in relation to algal toxins (Oregon Health Authority, 2019[27]). EPA has developed Section 304(a) criteria for algal toxins[28], the values contained in the Oregon Health Authority document are also at the same level as the EPA 304(a) criteria. Direct measurement of cyanobacteria cell counts is limited and currently prohibitively expensive at large scales. Until this method becomes more widely adopted or technology improves to reduce the cost, the Department will collect data on algal toxin concentrations as a surrogate indicator for cyanobacteria counts. While these toxin levels are recommended for protection of recreational uses, the Department is using these toxin levels as an indication that the phytoplankton community has experienced a significant shift in biodiversity. In balanced and diverse aquatic communities, cyanobacteria species are present, but are not in the quantities necessary to produce elevated toxin levels. Elevated toxin levels indicate that the biodiversity of the phytoplankton community is no longer balanced. In combination with an excursion of the nutrient screening thresholds, toxin levels above the recommended criteria indicate nutrient eutrophication is impairing the aquatic community.

[27] (https://www.sciencedirect.com/science/article/abs/pii/S0034425714002211)
[28] https://www.epa.gov/sites/production/files/2019-05/documents/hh-rec-criteria-habs-document-2019.pdf

- Cyanobacteria counts greater than 100,000 cells/mL suggest the presence and impact of a HAB in the water body. HABs and the algal toxins they produce pose a threat to the aquatic habitat protection and recreational designated uses. This data may be collected by agencies or county governments and, when available, the Department will request and use this information. The cyanobacteria cell count is based on the threat of unacceptable levels of algal toxins, which are currently being collected by the SLAP and the LMVP.

- Any algal toxin values exceeding the following thresholds during the same year one of the nutrient screening levels is exceeded will constitute evidence of impairment.

Microcystin	8.0 µg/L
Cylindospermopsin	15.0 µg/L
Anatoxin-a	15.0 µg/L
Saxitoxin	8.0 µg/L

d. 10 CSR 20-7.031(5)(N)6.D. – Observed shifts in aquatic diversity attributed to eutrophication

The health of an ecosystem can be assessed by looking at different aspects, one of which is the food web or chain. Chemical measurements can be taken to assess the nutrients and chlorophyll (as a surrogate for algae). Relative abundances of fish at the various levels of the food chain can be surveyed to see if it is in balance. High nutrient inputs along with high levels of suspended solids can cause a decrease in the number of sight-feeding predators and an increase in the number of the prey that the predators are unable to catch. More numerous prey put a strain on the resources available, resulting in smaller prey and smaller, less numerous predators. This imbalance in the number and/or size of fish, or a shift to less sight-feeding fish in favor of bottom-feeding fish such as carp, due to eutrophication is a cause for concern.

As the state agency responsible for the protection and management of fish, forest, and wildlife resources, MDC regularly monitors populations of primary sport fishes (black bass, crappie, catfish) in major reservoirs (typically annually) to ensure the agency has appropriate regulations in place to manage these fish populations for today and into the future. These populations of piscivorous (i.e., fish eating) sport fish, and the many planktivorous (i.e., plankton eating) non-sport fish that are their prey, are self-sustaining in Missouri's major reservoirs. Correspondence with MDC Fisheries Division confirms the agency does not conduct supplemental stocking for primary sport fishes (i.e., apex predators), nor does the agency conduct supplemental stocking of non-sport fish lower down the food chain (MDC, 2018).

Although MDC does not stock the primary sport and non-sport fishes noted above, MDC does stock additional fish species to provide a "bonus" or "specialty" sport fishing opportunity. Species included in the bonus or specialty fishing opportunities include (but are not limited to) paddlefish, rainbow trout, brown trout, striped bass, hybrid striped bass, walleye, and muskellunge. Many of these fish species are non-native and would not be capable of reproducing or sustaining populations in Missouri lakes.

MDC uses various sampling techniques including electrofishing, netting, creel surveys, and angler surveys to collect information related to fish populations and angler satisfaction over time. These data help to inform MDC's regulations for the capture of fish within Missouri lakes to ensure self-sustaining populations of sport- and non-sport fishes. The Department, in

consultation with MDC, will use these data to determine whether shifts in aquatic diversity attributed to eutrophication are occurring in a lake.

- The Department will request any available information on the potential biological shifts in fish or invertebrate communities related to eutrophication. This includes data from other agencies (such as the U.S. Fish and Wildlife Service) that monitor the populations of game fish.

- The MDC regularly monitors fish populations of primary sport fishes (black bass, crappie, catfish) in major reservoirs (typically annually) to ensure the agency has appropriate regulations in place to manage these fish populations for today and into the future. These populations of sport-fish, and the non-sportfish that are their prey, are self-sustaining in Missouri's major reservoirs.

- The MDC uses various sampling techniques including electrofishing, netting, creel surveys, and angler surveys to collect information related to fish populations and angler satisfaction over time. These data in consultation with MDC will be used to determine whether shifts in aquatic diversity attributed to eutrophication are occurring in a lake.

- The MDC produces annual fishery management reports for Missouri's major lakes and reservoirs that detail the health of the fishery and includes number of species, catch per unit effort, relative density of fish and measures of fish condition and population size structure. One such example of an annual fishery management report is the Stockton Reservoir 2017 Annual Lake Report (published March 2018). The data supporting MDC's annual fishery management reports can also be made available to the Department. MoDNR will request these annual reports and data from MDC.

e. 10 CSR 20-7.031(5)(N)6.E. – Excessive levels of mineral turbidity that consistently limit algal productivity during the period May 1 – September 30 (i.e., light limitations)

It is widely recognized that mineral turbidity reduces transparency and thereby limits algal production (Jones and Hubbart, 2011). Excessive mineral turbidity and reduced water column transparency can suppress Chl-a levels despite high levels of nutrients. Pronounced and extended turbidity events could have the effect of reducing Chl-a on an average annual basis but still allow for periodically high peaks or algal blooms after sedimentation of mineral turbidity and increased transparency. Under such conditions, waterbodies experiencing harmful algal blooms may go undetected when assessed as an average annual geomean. The intent of this response variable is to identify such waterbodies that might otherwise go unidentified as impaired.

There are several ways to determine light availability in a lake. Some examples include: Secchi depth, light attenuation and photosynthetically active radiation (PAR), Chl-a/TP ratios, and measurements for turbidity and suspended sediments. All these methods can provide additional information on the amount of light available in the epilimnion and how deep it penetrates into the lake. These data will be used to determine whether the lake has excess sediment in relation to nutrients for eutrophication impacts to occur.

- Excessive mineral turbidity can reduce light penetration within the photic zone of lakes and limit algal productivity due to the lack of sunlight. Water clarity can be expressed through

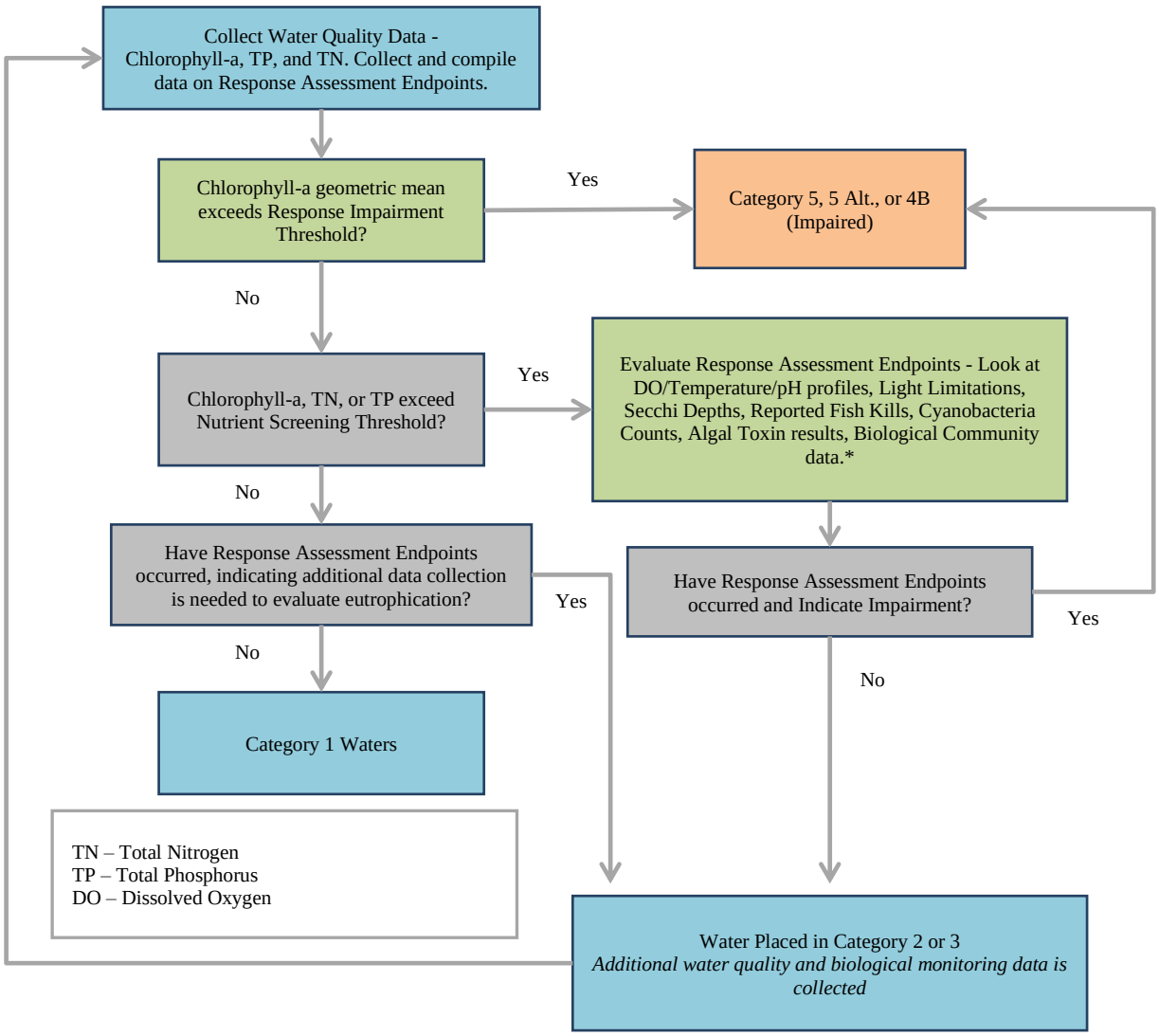
measurements such as Secchi depth, turbidity, and suspended solids. These data are collected by the SLAP and the LMVP under a cooperative agreement with the Department.

- Measured lake Secchi depths less than 0.6 meters in the Plains, 0.7 meters in the Ozark Border, and 0.9 meters in the Ozark Highlands is likely an indicator of excessive mineral turbidity that limits algal productivity in the water body (MDC 2012). This data is collected by the SLAP and the LMVP under a cooperative agreement with the Department. Yearly average Secchi depths below the applicable ecoregional value may constitute evidence of impairment. Additional analysis of average Chl-a/TP ratios will also be conducted before determining impairment status, as described below.

- The ratio of the average Chl-a to the average TP is an additional indicator of chlorophyll suppression in lakes due to mineral turbidity. A mean Chl-a/TP ratio less than or equal to 0.15 and a mean inorganic suspended solids value greater than or equal to 10 mg/L is suggestive of excessive mineral turbidity which limits algal productivity (Jones and Hubbart, 2011). Unless attributed to other physical factors, Chl-a/TP ratios at or below 0.15 and an ISS value greater than or equal to 10 mg/L as determined by yearly means will serve as an indicator of excessive mineral turbidity and constitute evidence of impairment. Assessment threshold values for Secchi depth, Chl-a/TP ratio, and ISS shall all be exceeded before determining a water is impaired.

- The Department will use data collected using a Li-Cor quantum sensor. Data collected with this equipment consists of light attenuation and photosynthetically active radiation (PAR). Until scientific literature on this new technology can be developed, the Department will rely on best professional judgment for when the data indicate light availability is limiting algal production to the point that if there were less or no limitation then the Chl-a values would be likely to exceed the criterion.

Figure 3. Missouri Ecoregional Numeric Nutrient Criteria Decision Framework based on the Bioconfirmation Approach.



Trend Analysis

The Department currently reports on physiographic region trends in Missouri's 305(b) Report. The latest version as well as past versions can be found on Missouri's impaired waters website: https://dnr.mo.gov/water/what-were-doing/water-planning/quality-standards-impaired-waters-total-maximum-daily-loads/impaired-waters. These trends have been reported every cycle in the 305(b) Report since 1990. Trends for the physiographic regions are calculated based on at least 20 years of data. Trends are developed for Secchi depth, total phosphorus, total nitrogen, total chlorophyll, nonvolatile suspended solids, and volatile suspended solids.

The Department will evaluate individual lake trends for total phosphorus, total nitrogen, and Chl-a. Nutrients and chlorophyll can be seasonally variable, as well as wet and dry weather dependent. A minimum of ten years of data will be necessary to confidently evaluate water quality trends in Missouri

lakes due to significant annual variability and differing hydrologic conditions. Longer time periods are needed for more accurate predictions of impairment.

- When evaluating trends, confounding, or exogenous variables, such as natural phenomena (e.g., rainfall, flushing rate and temperature), must be controlled for.

- The trend must be statistically significant. This process involves standard statistical modeling, such as least squares regression or Locally Weighted Scatterplot Smoothing (LOWESS) analysis. To be considered statistically significant, the p value associated with the residuals trend analysis must be less than 0.05.

- Impairment decisions based on trend analysis should, at a minimum, demonstrate that the slope of the projected trend line is expected to exceed the chlorophyll criterion within five years and that there is evidence of anthropogenic nutrient enrichment. If the slope of the projected trend line is expected to exceed the chlorophyll criterion in greater than five years, the lake will be prioritized for additional monitoring. A list of lakes that have increasing trends of nutrients or Chl-a will be added as an appendix to Missouri's future 305(b) Reports and can be used for prioritization of nonpoint source watershed management plans.